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C.-E. A. WINSLOW

It is characteristic of Science and Progress that they continually open new fields to our vision.—PASTEUR

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ALICE IN VIRUSLAND[1]

PAUL F. CLARK

Medical School, University of Wisconsin, Madison

(My apologies to Lewis Carroll with the hope that he will extend his usual gracious blessing to this latest parody on his immortal "Alice.")

PROLOGUE

Since this particular President has had only eight months in which to ripen instead of the usual twelve, you must expect his remarks to be a little green, possibly even somewhat tart. If they should prove too acid, I beg you to expose them to the warm California sun to supply sugar and a richer flavor. As a matter of fact, I wrote another address, "Immunity in Poliomyelitis," for this occasion, but it was so heavy that the thought of a long day of meetings in the summer followed by a sumptuous dinner decided me to discard the heavier in favor of a lighter medium; so I pray you, please bear with me and be generous. May I add for the benefit of the ignorant ones that my wife's name is also Alice and that she works at times with me in the laboratory. So if on occasion "our Alice" should make a grown-up remark you will realize that she has a multiple personality.

Alice was getting tired of waiting for her father; she was sitting in the one comfortable chair in the laboratory wondering *why grown-ups always had to finish things*, when suddenly a small monkey jumped out of the cage, dashed by her, and popped down the ventilating shaft. In a flash, down went Alice, trying her best to catch the runaway. Down, down, she went; it seemed like miles. The monkey was always just ahead of her and she

[1] Presidential address delivered before the Society of American Bacteriologists at its fortieth annual meeting, San Francisco, August 31, 1938.

could read his name "Ferdinand" on his collar. "I wonder," she thought, "if Ferdinand isn't going back to India to find his mother. Wouldn't that be fun! I should love to see that river they talk so much about, where the people all go to say their prayers. Let me see; is it the Styx or the Jordan? Oh no, I remember, it's the Ganges."

A blurred jumble of thoughts and hours passed as Alice hurtled on through the earth, when suddenly, ke-splash,—down she plopped into deep water. *Such* a douche! And *such* a plunge! But she swam hard, managing to come to the surface at last and to get to the shore just in time to see Ferdinand dash across the beach to the woods. After him raced Alice, only to see him jump into the trees, saying as he went, "Damn his tail and whiskers, I know I shall be late to the tea."

"That reminds me," thought Alice. "It *is* getting late and I *am so hungry*." As if they could read her thoughts, the monkeys threw down a shower of bananas from above, and Alice scrambled around picking up several. "Mmmmm, this is delicious!" said Alice, looking about at the foreign scene as she munched the ripe fruit.

"But what a curious feeling! I must be shutting up like a telescope!" She had eaten so heartily that the effect was immediate; by this time she was only three inches high. "I have heard of a bird's-eye-view," said Alice to herself somewhat frightened, "and I surely am getting one now. I do hope there is no cat about. He might be tempted."

She continued to shrink until she found herself looking in a neighborly fashion into the curious eyes of a large black ant who was comfortably puffing away at a briar pipe. "Curiouser and curiouser," thought Alice. "I suppose I am now getting an insect's view of things. I hope no bird will think me suitable for his afternoon tea," remembering with distress the ants she had sometimes trod upon. "I certainly shall watch my step when I get home again."

"How different one does feel when one is small and weak!" said Alice aloud.

"What do you mean,—small and weak?" asked the Ant, in a gruff voice. "Why, the strongest and most sensible creatures I

know are the smallest; just think of bacteria and the filterable viruses!"

Now of course, Alice, being her father's daughter, knew that bacteria and viruses cause many diseases, but she was astonished to hear the Ant discuss them as though they were rational beings. At a loss, she responded with the only polite remark she could think of, " 'Go to the ant, thou sluggard; consider his ways and be wise.' "

Whereupon the Ant, with a grin of pleased satisfaction on his bulbous face, removed his pipe and murmured, " 'Out of the mouths of babes and sucklings!' Who are you, anyway?"

ALICE IN CONVERSATION WITH THE BLACK ANT

Alice replied rather shyly, "I—I hardly know, Sir, just at present—at least I know who I was when I got up this morning, but I have changed so much since then—"

"Oh, so you're a changeling!"

Alice did not like this suggestion, so she turned away, absent-mindedly nibbling more of the banana which she still held in her hand. Rapidly she dwindled in size until she was smaller than the smallest grain of sand, and the Ant, her erstwhile neighbor, looked like a great irregular hill.

Viewing a world entirely new to her eyes, she was amazed by the minute, crawling, wriggling and tumbling creatures every-

where. The commotion around her, and the bumping of all the colloidal particles together caused loud crashing noises, so that she was terribly frightened and almost stunned by the roar. She managed, however, to find a small sand grain with jagged margins, so that with much effort, she pulled herself up out of the little damp spot where all these creatures were racing to and fro. She now began to look about her with more precision, and discovered out on the drier sand, away from the commotion at her feet, two strange creatures doing a peculiar dance. "What *can* they be?" she said aloud.

To her surprise, a rod-shaped creature with a large head, standing on the back part of *her* grain of sand answered, "What can be your name, and where do you come from, that you do not know two of the most powerful clans of our race?"

"Listen," said the Tetanus Bacillus, without waiting for Alice to answer, "and you shall hear what is troubling them."

THE COCCUS AND THE SPIROCHAETE

The Coccus and the Spirochaete
Were walking down the strand.
They choked upon a starfish;
"We simply cannot stand
Such large and clumsy proteins
Who think they are so grand."

"If trypsin strong could aid us now
By splitting him in twain,
Do you suppose," the Coccus said,
"T'would give us awful pain?"
"I doubt it," said the Spirochaete,
"There's too much tryptophane."

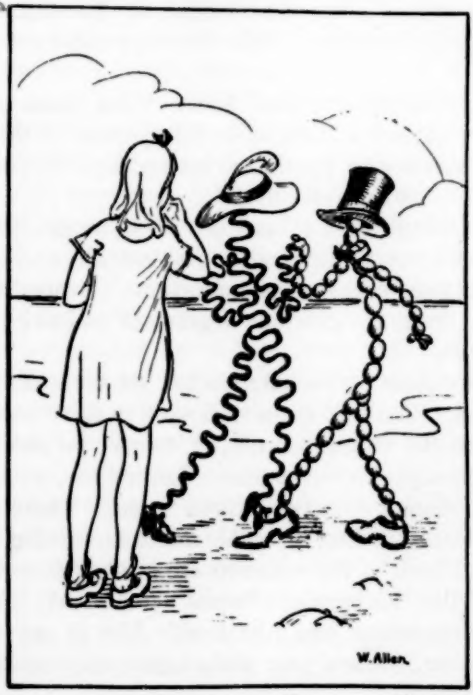
"The time has come," the Coccus said,
"To talk of many things,
Of stools and stains and stigmata
Of guinea pigs and goats.
And why the neutron has no charge
And whether iron floats."

Forward came the anaerobes
Moving in stately dance,
While spirochaetes on every hand
Wriggled a lively prance.
And all the little "viri" stood
Arrayed in purple pants.

"But wait a bit," the Coccus cried,
Deciphering his notes,
"This human race is surely mad
They slit each other's throats.
They blow each other off the earth.
They sink each other's boats."

"Should we permit," the Coccus asked,
"These silly beasts to thrive,
Who call themselves the lords of earth
Yet selfishly connive
Freedom, dignity and peace
From many lands to drive?"

Spake Influenza Virus then,
"Let's smite them hip and thigh,
They're clever, strong, and hard to beat,
But smack them in the eye.
If we combine in our attack,
Surely they must die."



THE COCCUS AND THE SPIROCHAETE ESSAYED A STATELY DANCE

"*Whatever do they mean?*" said Alice.

"Oh, they have called the Microbe Parliament together to consider what to do with 'man.' Come to the meeting with me this evening and you shall hear. Representatives from all the clans will be there."

"Will the king and queen be there?" asked Alice timidly.

"We deal not with dictators or kings," said Clostridium. "We be free people; free to gather, free to come and go, free to live our own lives without decrees from others."

As dusk came on, Alice and the Clostridium walked along, hand in hand, until they came with countless other strange beings to a great cave in the rock, with the roof seemingly supported by rows of stalagmites joining the stalactites from above. As they entered, Alice noticed that masses of small rods were gathered around each column and that each of these rods glowed from within with a pale greenish light, so that the whole cavern was beautifully illumined, with the rays reflected from every crystal.

"How very beautiful!" said Alice. "But these strange creatures! It is quite unbelievable!—Why, some of them look like parallelopipedons with peculiar arms and legs sticking out at all the corners! Are they all alive?"

"Alive?" said the tetanus bacillus. "Oh, we smallest folk never make that inaccurate distinction between life and death which you Gargantuans consider so important. Everything is alive. To be sure, there are different degrees of reactivity and of the excited states."

"Now, over there on the extreme left are some of the markedly excited clans. You call them radio-active substances. A little nearer us are the vitamins; only, of course, we don't give them your peculiar alphabetical names. Let me see, you have about fifty-seven different varieties, haven't you? There is Nicotinic Acid. See him, standing up on his nitrogen, waving his carboxyl tentacle. In front of the vitamins are,—what do you call them? —oh yes,—the hormones. Potent creatures! That clumsy fellow is Testosterone and just beside him is one of the chief causes of cancer. When your pathologists stop quarreling about

the origin of this and that malignant cell, and dig harder at the physiology of the cell, they'll get somewhere. But don't let them get so excited over what is animate and what is inanimate.[2] Take my word for it, that notion will go the way of the Ptolemaic Theory of the Universe."

"Oh," said Alice, in a very subdued voice.

"The only distinction *we* attempt to make is to keep the smaller, more active ones over here on the left where they won't disturb the more conservative clans, like my own over here on the right. See that trouble-maker sneaking over to the right; that's a haptene looking for a protein to give it a reputation. And do you see that most peculiar fellow way over at the extreme left, beyond the radio-active substances?"

"Yes", said Alice, "Why, it looks like a constantly changing interrogation point with a lot of funny arms sticking out everywhere. What is it?—or,—or, should I say, who is it?"

"Well," said Tetani, "that is man's best friend or his worst enemy, depending on your point of view. That is the virus of 'Satiable Curtiosity';—he doesn't stay around here very much, he really prefers the company of you Gargantuans."

Alice was almost overcome by the overwhelming scene, but Clostridium said they must hurry to their places as the meeting was about to begin. Straight up the central aisle to a rocky platform at one end, they marched, the Tetanus Bacillus leading the reluctant Alice by the hand. He seated her on a rose quartz crystal on the right of the stage.

In the center, seated on a large sapphire, was a peculiar-looking horseshoe-shaped animal which Alice recognized from her seashore experiences as that curious relic of past ages, the king crab. Her guide explained to Alice that Limulus was retained as chairman and judge of their proceedings because of his long line of illustrious ancestors and his *real blue blood*. "His family is almost as old as mine," said Tetani proudly.

Alice was speechless at the weird scene. Out on the floor of the cave was a great log jam of the strangest creatures. Opposite

[2] This idea is well presented by N. W. Pirie in an essay published in Perspectives in Biochemistry (1938) edited by Needham and Green.

her in an enclosure on the platform were twelve solemn-looking microbes, good and true, that appeared to be samples of the mass out on the floor.

"Most peculiar faces," muttered Alice. "That strangest moon-faced one with long whiskers must be the *Jabberwock*."

"Not at all," said Tetani, deeply offended. "That is Giardia lamblia, one of the wisest members of the jury."

"Silence in the court!" shouted the Anthrax Bacillus, standing up beside Limulus.

"Read the accusations," said Judge Limulus. Whereupon, the Streptococcus unrolled a parchment scroll on which were inscribed the itemized accusations against mankind:

"Through the skill of a clever member of the Bandar-log, we have with us this evening, a representative of the human race!" Pointing to Alice, he shouted, "She must answer to the charges!"

Alice buried her face in her hands, while the Coccus proceeded to list the accusations, most of which fell on her dazed mind as on deaf ears. She did, however, arouse herself sufficiently to hear the last few words as he summed up his appalling denunciation:

> "Brutality and blind-spots.
> Power to the panderers and the paranoiacs.
> Blind selfishness and blundering sentimentality!"

"Would you believe it," said the Coccus, "they build great structures for the care and preservation of the feeble-minded and permit these poorest strains to reproduce their kind!"

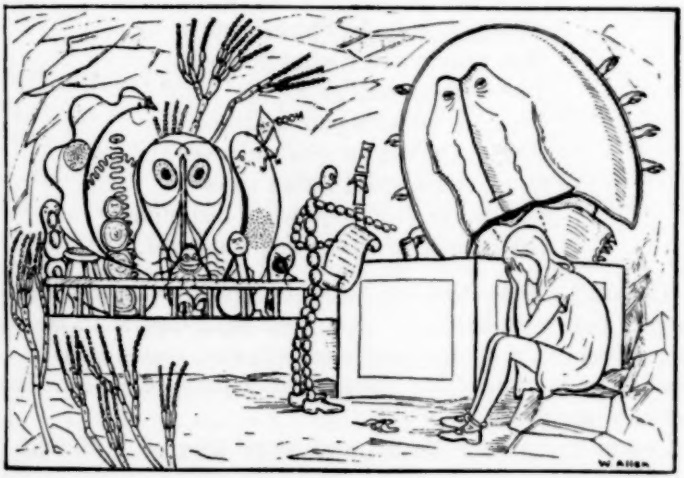
Alice continued to sit with buried face while the judge called for the evidence.

"For my part," said the Influenza Virus, "man seems too weak to worry about. With the help of members of some of our other clans, I destroyed millions of them a few years ago and I can do it again any time. War and resulting famine are our greatest aids, and their demagogic leaders easily inflame them to war. We have nothing to worry about. Man is foredoomed to destruction by his own folly."

"I am not so sure," said Corynebacterium diphtheriae. "My

family used to enjoy excellent accommodations in the throats of man, but recently, because of some vaccination procedure they have developed, we find it almost impossible to gain a throat-hold. Then, too, they heat their milk to such a temperature that our progeny are destroyed. It is a *'recession'* at least for us, although we *may* be able to meet it temporarily by 'inflation' while developing more virulent strains."

Eberthella typhi urged immediate action. "They heat their milk, they put some killing stuff in their water, and they have



THE HUMAN RACE, IN THE PERSON OF ALICE, BEFORE THE TRIBUNAL OF THE MICROBE PARLIAMENT

developed some way of destroying us inside their bodies. Why, in some cities, our immediate family no longer appears on their ledgers."

"Off with her head! Off with her head!" came the cry.

"And the bacteriophage will git you if you don't watch out" shouted somebody in the audience.

Spoke up the Streptococcus again, "I had intended to keep my people out of this discussion, because we have always been so powerful, but I am here to say that recently, man has devised a

cunning minute torpedo which 'gets' many of us. We do not feel so completely sure of ourselves as in former years. Maybe man is more capable than we used to think."

Treponema pallidum joined in. "Of course, you understand that for obvious reasons we have always had the inside track, but man has been sending stronger and stronger torpedoes after my relatives in recent years. If human beings really put their minds to it, I believe they could come *pretty close* to wiping out many of our *more highly-specialized* microbe families."

A chant arose from a portion of the audience:

"Wriggle, wriggle, Spirochaete,
How they wonder what you eat,
Down the vessels as you go
Bringing humans pain and woe.
Perhaps you favor glucose sweet
Or carotene you'd rather meet?
But watch your step, when histamine
Gives place to strong arsphenamine."

The next witness was the Tubercle Bacillus who said that his people, too, were hard put to it, and were unable to maintain themselves in the luxury their ancestors were accustomed to. "I second the motion of the Streptococcus that we all combine to elminate mankind."

Cheers from the audience and more cries of "Off with her head!"

Poliomyelitis virus spoke up, "That's all very well for you common laborers with no specialized training. But what would happen to my family if man should be eliminated? We should be wiped out, too. We live through man alone. It strikes me that you began this meeting on a very lofty plane—man should be eliminated for the welfare of the universe, because he is so stupid and immoral,—but what you really want is to save your own jobs and your own skins, just like all the politicians. As a matter of fact, Mycobacterium tuberculosis hominis and several others that have spoken would be in a bad way if man were eliminated. You are all just as selfish and short-sighted as is man. If you would

but learn to adapt yourselves to man's physiology, as *we* have almost succeeded in doing, and not destroy him, *he* would not develop means of eliminating you. He is not such a bad fellow if you know him as I do. I hold no brief for members of the human race. Of course, they are weak and selfish and gullible; like us, they are controlled by their emotions instead of their brains. Occasionally even some of their scientists drift over into mysticism and the metaphysical, and such a mess they make of it."

Chorus from the jury box:

"Oh let us never, never doubt
What nobody is sure about!"[3]

"Off with her head!" shouted some.

"Let her explain! Let her explain!" yelled others.

"She can't explain anything," bellowed the Judge, pounding the table for order. "I suggest that you consult the shades of some of the humans that have had sense enough to study *us*. They must surely be the wisest. Suppose two or three of you take Alice over to the Styx and see what some of those old fellows think of their species by now and what they think of your plan. There is one old codger[4] who sits all day in the sun squinting through a little hand magnifying glass and another long, skinny, bearded fellow, a recent arrival,[5] who looks through a big binocular microscope. See what they have to say."

Such an uproar broke out at this suggestion! Each microbe in the assembly tried to address the chair at once. "Just like the legislature!" thought Alice.

During the commotion, two of the least excited jurors came over, took Alice by the hand, and shuffled hastily out the back way, followed by the faithful Clostridium tetani. Outside, all four of them climbed on the back of an enormous fruit fly (Drosophila) who, at the word, sailed off across country with them.

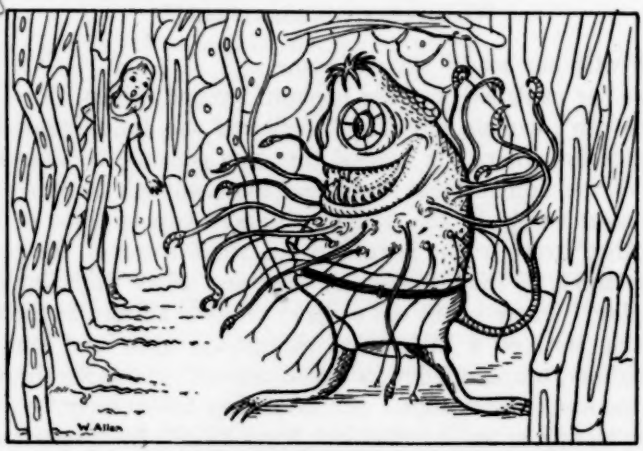
Alice heaved a sigh of relief to be free from the commotion of the trial, even though she knew nothing of her present destina-

[3] H. Belloc, 1925, J. Path. & Bact. **28**, IV.
[4] Antony van Leeuwenhoek.
[5] Theobald Smith.

tion save what the Judge had suggested. Never had she been so insulted and dragged about.

As they "whiffled" madly on "through the tulgey wood," she began to get her breath and to look about her.

Her two new neighbors were not at all reassuring. One looked much like her chief accuser, the Streptococcus, save that he had on a thick fluffy overcoat of the smoothest material; he sat up very straight and severe as if he were much impressed with his own importance. "He is a Type III Pneumococcus and very powerful," whispered Alice's mentor, Tetani. The other crea-



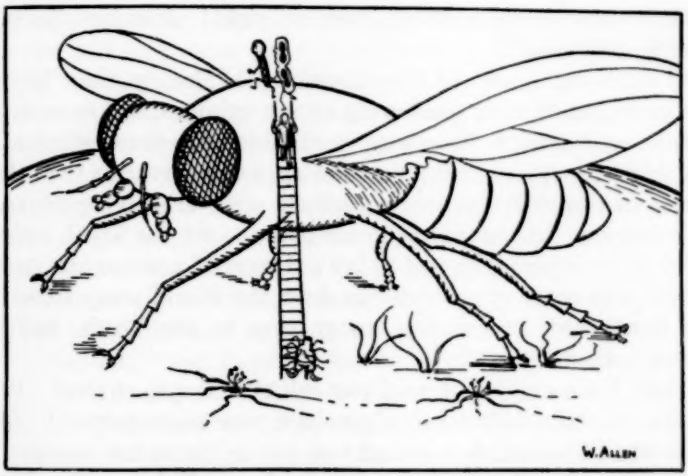
ALICE'S FIRST MEETING WITH A "VIRUS"

ture, Alice recognized as the Influenza Virus that had spoken so slightingly of the human race. He was a forbidding monster.

Four rows of talons lay beneath
Full twenty pair of canine teeth,
His fork'ed tail stuck out behind
With horny scales of every kind.
His eyes were red and white and green,
Changing with the shifting scene.
His nether limbs? You look askance!
Were covered still with purple pants.

"He is so ugly that he is really quite attractive," said Alice to herself, "but I should hate to get near him. With all those tentacles, he could seize dozens of me at once."

The Fly now landed them gently near a great river with many boats moored along the bank, and others going back and forth from shore to shore. "This must be the Styx," thought Alice. "Yes, it is, because on the deck of that nearest boat are the two old men the Judge was describing."



Air Transit: Virusland to the Styx

"Come," said Tetani, and together they climbed down a convenient spider web on to a pile of milkweed down. The Pneumococcus told Alice that she must interview a number of these scientists as the Judge had suggested. "You will drink some of the milk from this plant and that will make you grow to your normal size. The Tetanus Bacillus and I will be in your right and left ears respectively to tell you what to say and the Influenza Virus will be in your nose to bite you if you do not say the right thing."

Alice drank deeply of the milkweed juice and immediately began to grow in every direction. The Fly no longer seemed such

a huge creature, and soon she was looking *down* on ants and violets as on other days, before she chased the monkey down the ventilating shaft. Her vision, however, seemed much more acute than formerly.

"Ouch!" said Alice, as she felt a sharp nip in her nose, and heard a voice in her left ear bidding her go aboard the houseboat.

There she found the older man with a silvery wig, excitedly looking at something the tall, lean, bewhiskered fellow was demonstrating through his big binocular microscope.

"Marvelous! Wonderful! You *are* right! Just like the picture in this book!"

"I must say to you, as I've oft-times said already, that 'tis not my intention to stick stubbornly to my opinions, but as soon as people urge against them any reasonable objections, whereof I can form a just idea, I'll give mine up, and go over to the other side; and especially because my efforts are ever striving towards no other end than, as far as in me lieth, to set the Truth before my eyes, to embrace it, and to lay out to good account the small Talent I've received; in order to draw the World away from its Old-Heathenish superstition, to go over to the Truth, and to cleave unto it."[6]

"Yes, I see every coil, and you call that a spirochaete? But what a beautiful instrument of precision your microscope is! My little hand lens which I thought so fine is but a toy compared with this. But I did use my one talent. Tell me, has man improved himself as much as he has his optical instruments?"

"I fear not," said the slender one.

"The human race is in a rather delicate, unstable relation to its environment. Its course is frequently left to chance, in the hands of incompetents, instead of being guided by the coöperation of those best equipped. Only now and then is the voice of reason heard in the rare intervals when the human din subsides long enough for the race to catch its breath. The value of human life is profoundly distorted, and the untiring labor de-

[6] Letter to Rev. George Garden cited by Dobell—Antony van Leeuwenhoek and his "Little Animals," p. 74.

voted to attempts to save a single life is daily and hourly flouted on the highways. Important devices to protect community health, such as vaccination against small pox, are set aside because the accidental individual death rate resulting from their application may be in the second or third decimal place of a per cent."[7]

"Hmmmmm" whispered the Influenza Virus to the Pneumococcus. "He isn't overly enthusiastic about his human race, is he?"

"No," said the Coccus, "but they do make marvelous instruments and they write down their thoughts in books for all, who wish, to enjoy, even centuries later."

The Virus tweaked Alice's nose and suggested that she see who might be at the other end of the boat. Near the bow, Alice found a hawk-nosed, keen-eyed genial German Physiologist, Carl Ludwig, and a kindly, well-bearded old gentleman, a brother physiologist from Russia, Pavlov, poring over the description and the results obtained with the latest ultracentrifuge. "Grossartig —kolossal—ein Baustein!" cried Ludwig. "Die Methode ist alles! Die Methode ist alles!"[8]

"Yes, yes!" said Pavlov, "but consider the centuries in which this instrument was being developed." Turning to Alice, he said, "I beg you, bear this message to the scientists of your country—'Gradualness, gradualness, and gradualness. From the beginning of your work, school yourselves to severe gradualness in the accumulation of knowledge. Learn the ABC of science before you try to ascend to its summit. Never begin the subsequent without mastering the preceding. Never attempt to screen an insufficiency of knowledge even by the most audacious surmise and hypothesis. Howsoever this soap-bubble will rejoice your eyes by its play, it will inevitably burst and you will have nothing except shame."[9]

[7] Parasitism by Theobald Smith, Princeton Press, 1934, p. 170.

[8] "Die Methode ist alles" was a favorite thesis of Ludwig.

[9] A part of Pavlov's Testament to the youth of his country written just before his death, 1936.

Much impressed, the Coccus remarked to the Influenza Virus, "These Gargantuans with their written language, the high ideals of their philosophers and scientists, and their printed books, must surely be more effective than we can ever hope to be. They should be able to inspire and teach everybody."

"Humph!" said Influenza Virus. "Did not the 'lean one' say that their leaders were frequently incompetent? We can divide and multiply, can't we?"

"Yes, but we have no enterprise, no memories of the past, no record of the achievements of great individuals of power and spirit to stimulate the whole race."

"We can work the more effectively," grunted the Virus.

Said the Coccus, "We can accomplish great tasks, but we are not individuals; we are merely masses. We do not even know our own parents."

"Pooh," said the Virus. "Do you see those two men over on the next boat? Well, that fellow with the van Dyke beard is the greatest poet and seer of them all; he states of mankind that 'It is a wise father that knows his own child.' And a keen woman essayist from Philadelphia, Agnes Repplier, puts it even more sharply when she says that 'maternity is a matter of fact, while paternity is a matter of conjecture.' Even Lantlsteiner's contributions and skill merely narrow the limits of the conjecture."

Alice interrupted the dispute by saying, "I recognize Shakespeare, but who is the other man with the large nose, the shining eyes, and the thin face? He is almost surrounded with books. Did he write all of them?"

"Yes," said the Coccus, "that is Erasmus, the apostle of the 'book,' of fair-mindedness, of conciliation, and the master satirist and enemy of war and of fanaticism. He lost the struggle in his own period and he certainly would lose it today. But he knew that 'Fanaticism is fated to overreach its own powers. Reason is eternal and patient, and can afford to bide its time.'[10] But the leaven of reason must permeate the whole people in order to be effective."

[10] Erasmus of Rotterdam by Stephan Zweig, Viking Press, 1934, p. 21.

At that moment, a great bell sounded. Pavlov put down his book saying it was tea-time, and would Alice be so kind as to be their guest; Alice accepted with alacrity. Leisurely they sauntered along, other shades joining the group from each of the houseboats, all making their way to a large central building.

As they went inside, Alice was delighted to see Ferdinand, the monkey, seated at one of the tables. She darted over, and slid into the chair next to him. "So you aren't going to be late to the tea," said Alice.

"Oh, that was yesterday," said the monkey, "this is tomorrow."

"At any rate," said Alice, "apparently we have jam this week," as she took a huge slice of bread and butter spread thickly with strawberry preserves. While munching this, she looked about her with amazement. At one end of the table was Puck, and at the other, the Mad Hatter. All of her recent acquaintances were there, and across the table was Pasteur dilating to Lord Lister on the value of laboratories to the human race, and right next to Lister were Tweedle Dum and Tweedle Dee.

On her right, Erasmus and the Unknown Soldier were discussing methods of preventing war. Said Erasmus[11], "One war springeth of another. . . . There is agreement among poisonous serpents. But unto man, there is no wild or cruel beast more hurtful than man. . . . Moreover, when the brute beasts fight, war is one for one, yea and that very short. . . . When was it ever heard that an hundred thousand brute beasts were slain at one time fighting and tearing one another? . . . Now if man will weigh, as if it were in a pair of balances, the commodities of war on the one side and the incommodities on the other side, he shall find that an unjust peace is far better than righteous war. In war, the most part of the punishment and harm falls upon them that least deserve to be punished, that is, upon husbandmen, old men, honest wives and young children. There is no tongue can tell the harm and mischief that we feel in war." The Unknown Soldier gravely nodded his head in complete assent, then said that he had written a few verses that expressed his hopes and dreams.

[11] Erasmus, "Against War."

THE DREAM OF THE UNKNOWN SOLDIER

The filth and squalor, the lice, the stench,
The shrieking of shells, death in the trench,
To me are but memories far in the past.
Would that the World War *had* been the last!
A dream have I for the "brave new world"
Of beauty and truth, with war banners furled.

Sweet grapes, fresh milk, hot bread and roast meat,
Soft wind on cheek, damp grass on bare feet,
Sunset and skies, deep woods and swift brooks,
Friends and kind words, music and books,
Work and fatigue, achievement with zest,
Home after storm, and well-earned rest.

Ample for all, and none with too much,
With leisure for painting, 'cello, and such.
No excess of power, no surfeit of wealth,
"No one is free who rules not himself."[12]
Full scope for each, both humble and brave
With teachers of spirit from cradle to grave.

This free-will choice, each man must make
'Twixt growth of self, and selfish sake.
Beauty is near for all to enjoy,
But truth lies hidden in hard alloy.
Humane ideals, not lust for power
Must rule, if the World would truly flower.

"I'm afraid I don't quite understand," said Alice timidly. Neither did Ferdinand for he grabbed two bananas from the table and darted for the door. Alice made after him, intending not to let him get away from her this time. But she stubbed her toe on the doorsill and felt herself falling, falling, not to the floor, but into her father's arms.

"It is time to wake up, Alice," said her father. "You have had quite a nap."

"Oh—where's Ferdinand,—where's the monkey?" said Alice, rubbing her eyes. "I've had the strangest dream, all about viruses and people and everything. I must tell you all about it."

"Well, it is time to go home to supper now, and then you may continue your dreams. For tomorrow we start bright and early

[12] Epictetus.

for the unscanned western mountains, and the distant Golden Gate.—Do you think we shall get there?"

"Of course," said Alice dreamily. "But it will take a long time."

"Yes," said her father. "It will take many centuries."

EPILOGUE

"He who remembers what man is, can be discontented at nothing which happens."[13]

[13] Epictetus. This epilogue is thought to be in harmony with the philosophy of the paper but not with the spirit of the Annual Banquet, so it was not read at the meetings.

SCIENTIFIC PROCEEDINGS

FORTIETH GENERAL MEETING OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

SAN FRANCISCO, CALIF., AUGUST 30, 31 AND SEPTEMBER 1, 1938

Headquarters: Hotel Fairmont

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President, PAUL F. CLARK, University of Wisconsin, Madison, Wis.
Past-President, JAMES M. SHERMAN, Cornell University, Ithaca, N. Y.
Vice-President, ARTHUR T. HENRICI, University of Minnesota, Minneapolis, Minn.
Secretary-Treasurer, IRA L. BALDWIN, University of Wisconsin, Madison, Wis.

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ABSTRACTS*

Report of the Committee on Bacteriological Technic: Progress during the First Half of 1938. H. J. CONN, *Chairman;* J. H. BROWN, VICTOR BURKE, BARNETT COHEN, M. W. JENNISON, J. A. KENNEDY, AND A. J. RIKER, *Members.*

This report covers the work of hardly more than six months, for the last report of the Committee on Bacteriological Technic was presented in December, 1937. During this period the Committee has revised *Leaflets VI* and *IX*, and the *Index* of the *Manual of Methods for Pure Culture Study of Bacteria.*

Sales of the *Manual* have been increasing rapidly during the last few years; so much so that in 1937 receipts from the sales of this publication were $170 larger than any preceding year, except 1923, when the *Manual* was first issued. During the first five months of 1938, moreover, receipts have been $114 larger than during the same period of 1937. The subscription list to *Pure Culture Study of Bacteria,* the continuation service for the *Manual,* has likewise been growing during this same period.

The sales of the *Descriptive Chart* have not been increasing, and it is felt that the latest revision of the *Chart* may have made it too complicated for use in teaching. Accordingly, a preliminary draft of a new and much simplified *Chart* is now being submitted to the Society for consideration. It is intended for use in instruction only and it is planned to supplement, not to replace, the present *Chart.* Although it is not to be put on sale for some time, anyone interested may receive a copy of the suggested form by application to the Chairman of the Committee or to Dr. M. W. Jennison of the Massachusetts Institute of Technology. Comments and criticisms from anyone inspecting a preliminary copy will be welcome.

Report of the Committee on Biological Abstracts. A. P. HITCHENS, *Chairman.*

It will be remembered that *Abstracts of Bacteriology,* supported by

* This number of the JOURNAL has been edited by the Chairman of the Program Committee. Authors of the abstracts in the Scientific Proceedings have not seen proof, due to restrictions of time imposed by the printing schedule.

this Society as one of its official organs for 9 years, was merged into *Biological Abstracts* in 1925. Since then, *Biological Abstracts* has been functioning continuously as a reviewing and indexing periodical for all of the biological sciences.

As a result of circumstances which are too well known to need discussion here, it has become necessary for biologists themselves to finance *Biological Abstracts*, if its publication is to be continued. Toward the end of last year an emergency plan was developed which has permitted publication of this year's volume. The time has now come to discuss permanent support for *Biological Abstracts*, and it is my belief that the Society of American Bacteriologists has an opportunity to procure whatever the membership may desire in the way of an abstracting journal.

Up to the present time, it has been necessary for any subscriber to purchase each annual volume of *Biological Abstracts* in its complete state covering all the phases of biology. Some bacteriologists have felt that this was wasteful, since their interests did not extend so broadly. The present board of trustees of *Biological Abstracts* is willing to change this policy. If bacteriologists and immunologists are interested, there is a chance to have *Biological Abstracts* split into sections. Our section would cover bacteriology, immunology, mycology, etc. The price per volume of the section would not be more than $5.00 per year. On the other hand, a subscriber to such a section would receive the annual index to the entire publication.

Another possibility, which to me is even more attractive, is that our Society, through an interested committee, may practically assume the editorial management of its particular section. The editor of *Biological Abstracts*, and his associates, are so coöperative that they are willing to make any practical arrangement with the Society of American Bacteriologists for publishing such abstracts of current bacteriological literature as may be furnished to them by the committee. Furthermore, such abstracts will be published promptly in accordance with the announced policy of having in print abstracts of all biological literature readily available within 2 months of the original publication.

Many other things might be said about *Biological Abstracts* and its value to bacteriologists. An abstract journal seemed so necessary to us a few years ago that we were willing to support such a publication all by ourselves. That publication left many things to be desired, but now we have the means offered to us for doing an entirely satisfactory job. In other words, if there are in our membership individuals who care to assume responsibility for getting out the abstracts promptly, the

copy will be published promptly and indexed thoroughly and carefully. In all of these matters, the editorial office of *Biological Abstracts* will meet a committee of our Society at any point and carry on from there.

It is my belief that a plan can be worked out, and that the result will be attractive to bacteriologists everywhere. It will in effect give us back our *Abstracts of Bacteriology*. Then, I believe, a sufficient number of our members will add their names to the subscription list to render effective aid to a worthy project.

I am well aware of the fact that a portion of our members feels no need for a review journal to keep in touch with developments in the biological sciences. Chiefly, such persons are in large teaching and research institutions. Such individuals would seem to have no place on a committee, whose function would be that of seeking to aid less fortunately situated members in keeping abreast with current developments. May I suggest, therefore, the appointment of a committee of persons interested in an abstracting journal, persons who feel the need of it as a research instrument and who are willing to devote time and thought to the formulation of a plan by which our Society may cooperate with the editorial board of *Biological Abstracts*?

GENERAL BACTERIOLOGY

G1. Nutrient Requirements of Butyric-Acid Butyl-Alcohol Bacteria. R. W. Brown, H. G. Wood, and C. H. Werkman, Tuskegee Institute, Tuskegee, Ala., and Iowa Agricultural Experiment Station, Ames.

Experiments were made to determine the nutrient substances essential for the dissimilation of glucose by butyric-acid butyl-alcohol bacteria. Eight cultures, including strains of *Clostridium acetobutylicum*, *Clostridium butylicum*, *Clostridium pectinovorum*, *Clostridium felsineum* and *Clostridium beijerinckii*, were used. The organisms were grown in tubes containing 10 cc. of medium composed of various combinations of hydrolyzed casein, ammonium sulfate, acidic ether-soluble extract of yeast extract (Difco), vitamin B_1, tryptophane, Speakman's salts, mono- and di-basic potassium phosphate and glucose. Speakman's salts were composed of K_2HPO_4, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, NaCl, $FeSO_4 \cdot 7H_2O$ and $MnSO_4 \cdot 4H_2O$. Growth was measured by the quantitative determination of glucose, before and after fermentation, using a modification of the Munson and Walker method.

In the presence of ammonium sulfate, an acidic ether-soluble extract

of yeast extract (Difco) and Speakman's salts were essential for the vigorous growth of all of the organisms tested. Vitamin B_1 and tryptophane were not essential. Neither Speakman's salts minus phosphates nor the phosphates without the other inorganic salts were sufficient for growth in successive transfers.

With purified, hydrolyzed, vitamin-free casein as the source of nitrogen either the ether-soluble extract of yeast extract (Difco), Speakman's salts or phosphates alone were essential for growth in successive transfers. Speakman's salts minus the phosphates were not sufficient for continued growth. Tryptophane and vitamin B_1 were not essential.

Fermentations yielding the normal end-products from the dissimilation of glucose were obtained in both ammonium sulfate and hydrolyzed casein media when the other essential nutrients were present.

G2. The Utilization of Carbon Dioxide by the Propionic Acid Bacteria. H. G. Wood and C. H. Werkman, Iowa Agricultural Experiment Station, Ames.

Carbon dioxide supplied in the form of $NaHCO_3$ or $CaCO_3$ is utilized by propionic acid bacteria in the fermentation of glycerol. Utilization of CO_2 is accompanied by a molar equivalent formation of succinic acid. It is suggested that succinic acid may arise by synthesis from 3- and 1-carbon compounds.

Carbon dioxide uptake has been investigated in the Warburg respirometer, using non-proliferating cell suspensions or dry cell preparations in phosphate buffer and under an atmosphere of CO_2. Dissimilation of glycerol, erythritol, adonitol, mannitol, rhamnose and xylose showed an uptake of CO_2. Glyceraldehyde, dihydroxyacetone, arabinose, galactose and glucose were fermented with formation of no or very little CO_2, but under nitrogen significant amounts were formed. The implication is that the utilization of CO_2 increases with CO_2 concentration. The uptake of CO_2 or the fermentation of glycerol is not inhibited by KCN, 0.1 per cent; arsenite, 0.01 M; malonate, 0.03 M; pyrophosphate, 0.02 M; and sodium azide, 0.002 M. However, NaF, 0.00125 M, inhibits the utilization of CO_2 in glycerol dissimilation and increases the output of CO_2 from glucose. The increased yield from glucose probably is caused by the inhibition of CO_2 uptake. Although a net decrease in CO_2 has not been obtained as yet for all substrates, it seems probable that CO_2 may be utilized in most, if not all, dissimilations under the proper conditions.

G3. Studies on the Relationship between Synthesis and Respiration. C. E. CLIFTON, Stanford University, Calif.

Recent studies by the author show that the oxidation of acetate, butyrate or lactate is not carried to completion by well washed suspensions of *Escherichia coli*. The results suggest that a portion of the substrate is oxidatively assimilated by the cells and that the assimilatory process is blocked in the presence of suitable concentrations of sodium azide or 2:4 dinitrophenol, the oxidation then proceeding to completion.

These studies have been extended to include the oxidation of fumarate, succinate, glucose and a number of amino acids by well washed suspensions of *Escherichia coli* in phosphate buffer or in a saline-bicarbonate solution in equilibrium with a 5 per cent CO_2-95 per cent O_2 gas-mixture. The consumption of O_2 and the production of CO_2 were determined at 30°C. by the Warburg technic. The extent to which a given compound is oxidized has been found to vary markedly with the nature of the substrate, being approximately 75 per cent for acetate and 50 per cent for glucose. With the majority of compounds tested, it approached 100 per cent in the presence of suitable concentrations of sodium azide or 2:4 dinitrophenol.

Studies on the extent of oxidation of lactate or glucose during the growth of *Escherichia coli* in an inorganic medium gave results similar to those obtained with the washed suspensions. These, and other results which will be discussed, suggest that the respiration of heterotrophic bacteria may well be an oxidative assimilatory reaction which may be represented by the general equation:

$$\text{Substrate} + O_2 \rightarrow (CH_2O) + CO_2 + H_2O$$

This is much like the reaction which represents the assimilatory process in the green plant:

$$CO_2 + H_2O \xrightarrow[\text{energy}]{\text{radiant}} (CH_2O) + O_2$$

G4. Occurrence of Glutathione in Microörganisms. T. E. MILLER AND R. W. STONE, Pennsylvania State College, State College.

Glutathione, the sulfhydryl-bearing tripeptide associated with cell respiration, was quantitatively determined in various bacteria, yeasts and molds by use of the iodate-titration method of Quensel and Wachholder. The nitroprusside test was used as a qualitative check, and in practically all of the experiments it correlated with the quantitative determination. Members of the following genera showed determinable

amounts of glutathione in the cell: *Aerobacter, Escherichia, Proteus, Chromobacterium, Serratia, Rhizobium, Azotobacter, Lactobacillus, Saccharomyces, Torula, Penicillium, Aspergillus, Rhizopus,* and *Monilia.* Four species of *Clostridium* had relatively small amounts; the cocci and members of *Bacillus* showed negative or questionable quantities. In comparison with normal human blood which has been reported as containing from 34 to 47 mg. of glutathione per 100 gm. sample, *Aerobacter aerogenes* contains about 27; *Proteus vulgaris,* 29–31; *Chromobacterium violaceum,* 7–45; baker's yeast, 59; *Torula sp.,* 57; and *Monilia sitophilia,* 20–38 mg. per 100 gm. of moist organisms.

Both aerobes and anaerobes contain the tripeptide, thus there is no apparent correlation of glutathione content with anaerobiosis. The data indicate that glutathione is an intracelllar substance, since the culture medium usually contained only from 2 to 20 per cent as much of the peptide as the organisms. As glutathione is the coenzyme of methylglyoxalase, these results lend support to the theory that it is possible for methylglyoxal to act as an intermediate in most microbiological fermentations.

G5. The Effect of Certain Amino Acids and Ascorbic Acid on the Growth of Staphylococcus aureus in Casein Solutions. LIONEL FARBER, Hooper Foundation for Medical Research, University of California, San Francisco.

A study was made of the growth of a strain of *Staphylococcus aureus* in a 1 per cent casein-inorganic salt medium to which were added varying quantities of the substances to be tested. The growth was measured by determining the total nitrogen in the washed bacterial centrifugate. The following amino acids separately stimulated the development of the organism: *d*-glutamic acid, *d,l*-histidine, *d*-arginine, *l*-proline, *l*-hydroxyproline, *l*-cystine and *l*-cysteine. Ascorbic acid also had a growth-promoting effect. The increases varied with the individual substance and the concentration. Extracts of beef liver, spleen and kidney, of hog pancreas and small intestine, and of yeast elicited more marked growth-enhancing actions than any one of the above materials.

G6. Certain Factors Affecting the Growth of Yeast. NATHAN F. TRUE, LLOYD PAUL, NORMAN J. MILLER AND PAUL S. PRICKETT, Mead Johnson and Co., Evansville, Ind.

The enumeration and control of yeasts are important problems in industries other than brewing and distilling. These problems, particu-

larly rapid methods of enumeration, are dependent on factors affecting the growth of yeast. Bacterial growth interferes with such methods and may cause confusion unless controlled.

In both liquid and solid media the following factors were found to be important: composition of nutrients in the medium, concentration of carbohydrates, hydrogen ion concentration, atmospheric oxygen, and fractions of the vitamin B complex. It appears, however, that various types of yeast are influenced more markedly by some of these factors, either singly or in combination, than are other types.

The following results are typical. Malt syrup was plated on Wort Agar (Bacto) and incubated for 4 days at room temperature to afford a basis of comparison. The yeast plate count was 20 per gm. of syrup. Various dilutions of the malt syrup ranging from 10 to 50 per cent were prepared with sterile water. In the plain malt syrup-water dilutions bacterial growth was a complication. One-tenth per cent of thiamin chloride (vitamin B_1) accelerated the growth of yeast but did not check the bacteria. Adjusting the pH to 4.7 did control the bacteria. A 30 per cent malt syrup concentration, adjusted to pH 4.7 and containing 0.1 per cent of thiamin chloride, gave typical gas production and characteristic odor of yeast fermentation in 56 hours. The diagnosis was confirmed microscopically.

In a series of tests on different malt syrups, the above method has proved to be more sensitive as well as more rapid.

G7. The Cultivation of Bacillus coli and Bacillus typhosus on Synthetic Media Containing α-Alanine, l-Cystine, l-Tryptophane and d-Threonine as Sources of Nitrogen. M. H. Soule and Helen Loomis Gehring, University of Michigan, Ann Arbor.

A basic medium was prepared as follows: doubly distilled water, 1,000 cc.; glucose, 1.0 gm.; $MgSO_4$, 0.01 gm.; $CaCl_2$, 0.01 gm.; K_2HPO_4, 1.0 gm.; KH_2PO_4, 1.0 gm. It was sterilized in the autoclave at 120°C. for 15 minutes or by passage through Berkefeld W filters. The final pH was 6.8. The organisms under consideration would not grow on this medium. Individual lots, therefore, were enriched by the addition of a single amino acid, as follows: α-alanine, 1.0 per cent; *l*-cystine, 0.038 per cent; *l*-tryptophane, 0.1 per cent and *d*-threonine, 0.1 per cent. *Bacillus coli* grew luxuriantly on the medium containing the α-alanine. *Bacillus typhosus* failed to multiply. A period of adaptation was necessary before *Bacillus coli* grew well on the medium containing *l*-cystine. *Bacillus typhosus* failed to grow initially on the cystine-containing

medium, but an increase in the pH to 7.4 by the addition of K_2HPO_4 plus 0.0002 per cent of ferric chloride permitted growth. Growths of *Bacillus coli* were obtained on the medium containing *l*-tryptophane, provided ferric chloride were present, and on the pabulum containing *d*-threonine. *Bacillus typhosus* grew in the presence of *l*-tryptophane plus iron, although the addition of sodium lactate with gradual withdrawal was essential.

Several strains of *Bacillus coli* and *Bacillus typhosus* have been maintained by serial subculture at five-day intervals on the aforementioned media for over 100 serial transfers. Other amino acids have been substituted with negative results. Transfers to the usual differential media showed that the cells had not been fundamentally changed. Contrary to the reports in the literature, tryptophane did not seem to be essential to the growth of *Bacillus typhosus*.

G8. Metabolic Studies of a Non-Hemolytic Streptococcus. J. W. KING, J. C. GAREY AND M. A. FARRELL, Pennsylvania State College, State College.

A study was made of the effect of casein-acid digests, and the Dakin butyl alcohol fractions from these digests, on the growth of a non-hemolytic streptococcus. Under the conditions of this study, when the hydrolysate is fractionated according to the butyl-alcohol method of Dakin, it has been shown that the growth-stimulating properties of the hydrolysate are present exclusively in the butyl alcohol-insoluble fraction. This fraction contains the diamino and dicarboxylic amino acids.

The monoamino, monocarboxylic fraction exerts no effect on the growth of the test organism. The proline fraction, soluble in moist and dry butyl alcohol, actually inhibits growth. This inhibitory factor acts in extremely small concentrations and has not been identified. Inasmuch as the unfractionated digest does not evidence this effect, it is assumed that the process of fractionation and of purification altered this part of the digest in some way.

In an attempt to substitute various amino acids for the activating fraction, no single amino acid was found which entirely replaced the butyl-alcohol-insoluble fraction. The amino acids examined may be listed as activating, inhibitory or exerting no effect. Lysine, all monoamino, monocarboxylic acids, except alanine and α-amino valeric acid, and including the sulfur-bearing amino acids, exert no effect. Glutamic acid, histidine, arginine, alanine, and α-amino valeric acid activate. Tryptophane is the outstanding inhibitory substance. Growth was

measured by photometric readings, which are believed to be more accurate than visual estimations of turbidity, Nesslerization, or micro-Kjeldahl procedures.

G9. Cultural Requirements for the Production of Black Pigments by Bacilli. FRANCIS E. CLARK AND NATHAN R. SMITH, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

The conditions required for the blackening of media by 38 cultures of aerobic, spore-forming bacilli were studied. It was found that *Bacillus niger* produces black pigment upon protein media which contain free or metabolically available tyrosine. Some commercial peptones containing no readily available tyrosine are not blackened unless free tyrosine is added. The addition of a fermentable sugar to many protein media normally blackened by *Bacillus niger* prohibits pigmentation. *Bacillus aterrimus* blackens media containing fermentable carbohydrates, either in the presence or absence of tyrosine, but does not blacken sugar-free peptone media readily blackened by *Bacillus niger*. Carbohydrate media containing mineral nitrogen are blackened by *Bacillus aterrimus*, but not by *Bacillus niger*. Cultural differences between *Bacillus aterrimus* and *Bacillus niger* are believed sufficient to warrant their recognition as distinct species. *Bacillus tyrosinogenes* of Rusconi and *Bacillus nigrificans* were found similar in all respects to *Bacillus aterrimus*, and are believed to be synonymous with that species. *Bacillus betanigrificans* produces black coloration in the presence of iron, but not upon the media blackened readily by the species considered above. *Bacillus betanigrificans* possesses morphological and cultural differences that separate it from *Bacillus niger* and *Bacillus aterrimus*.

G10. Molds as Protein Food in Diets. C. E. SKINNER AND EUGENE MULLER, University of Minnesota, Minneapolis.

Aspergillus oryzae, Penicillium roquefortii, Penicillium flavo-glaucum, and two unidentified species of *Penicillium* were grown on media composed of inorganic salts, water, and sugar. The dried, ground mycelium was fed to rats as the sole source of protein in otherwise complete diets. The only other organic nitrogen consumed was 100 mg. of yeast-extract powder fed daily. Paired-animal experiments showed that a slow growth-rate was maintained, but that methionine was present in minimal amounts, since a statistically significant stimulation to growth was evinced when 0.25 gm. cystine or methionine, but not when any other amino acid tested, was added to 100 gm. of ration. The molds syn-

thesized from inorganic-nitrogen complex compounds indispensable for animal growth as lysine, valine, tryptophane, histidine, phenylalanine, leucine, iso-leucine, α-amino-β-hydroxy-n-butyric acid, and small amounts of methionine. Positive Millon reactions showed tyrosine or other phenolic compounds to have been synthesized. When maize or wheat was cracked, moistened, sterilized, and inoculated with *Penicillium flavo-glaucum*, allowed to become very moldy, and dried, there was no significant difference between moldy grain as a protein source in diets and similar grain so treated but uninoculated. This was true whether or not the protein content of the moldy grain was brought to the level of the uninoculated grain by the addition of starch. The mold did not synthesize sufficient tryptophane or lysine to overcome significantly the amino acid deficiencies of these grains.

G11. Observations on Marine Anaerobes in Oval Tubes. CLAUDE E. ZOBELL AND D. Q. ANDERSON, Scripps Institution of Oceanography, University of California, La Jolla.

Thin-walled oval tubes, 6 x 14 mm. in cross-section and 380 mm. long, have proved useful for the enumeration and isolation of anaerobic bacteria, as well as for studying their metabolism and E_h requirements. The application of such tubes has revealed that sea water contains from one to nearly a hundred anaerobes per cc. Most are facultative anaerobes capable of growing over a wide E_h range. The anaerobic population of bottom deposits ranges from a few to over a million per gm. of mud. The total number of anaerobic bacteria decreases with core depth, but the proportion of strict to facultative anaerobes increases.

The ease with which oxygen can be excluded from the long narrow tubes facilitates poising the media at the desired E_h values by means of appropriate reducing or oxidizing agents. Microelectrodes have been perfected for making continuous measurements of the E_h and also the pH of different strata in the oval tubes. Most of the strict anaerobes from the sea start to multiply around E_h 0, although the optimum for their multiplication is between E_h −150 and −250 millivolts. They generally reduce the oxidation-reduction potential to about E_h −350 millivolts, although potentials as low as E_h −580 millivolts have been observed.

By using selective media, the oval tubes have proved useful for estimating the relative abundance of different physiological types of anaerobes (provided they do not produce gas). Special attention has been given to the study of lipolytic anaerobes isolated from the sea.

G12. The Presence in the Sea of Bacteria Belonging to the Genus Clostridium. MARGARET HOTCHKISS, Oceanographic Institution, Woods Hole, Mass.

The examination of marine samples for the presence of members of the genus *Clostridium* has confirmed the findings of a preliminary study reported in 1933 from the Woods Hole Oceanographic Institution. Organisms having the morphology of *Clostridium* were found in "bottom-material" and were most abundant near shore, where decomposable substances were available. Samples of water taken from near the shore also showed the organisms. Diatom "tow" afforded a site where the organisms could thrive. The organisms fermented glucose with the production of gas and of butyric acid. Mixed cultures were able to grow in a nitrogen-free medium and to fix nitrogen. The organisms have been isolated in pure culture. In morphology they resemble members of the genus *Clostridium* commonly present in soil. Growth on artificial media has been very scanty.

G13. Physiological Events during the Dissimilation of Carbohydrate by the Living Bacterial Cell. C. H. WERKMAN, Iowa Agricultural Experiment Station, Ames.

A general review will be presented of the comparative behavior of the bacterial cell with respect to its aerobic and anaerobic dissimilation of glucose. In the light of present knowledge, an attempt will be made to reconstruct the physiological events in the living bacterial cell, particularly with respect to the transformations undergone by carbohydrate. The discussion will include a consideration of dehydrogenase systems, principal and subsidiary respiration, and final transformation of the intermediates by the bacterial cell.

G14. Recent Observations on the Enzymic Hydrolysis of Fats and Other Esters. A. K. BALLS, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

Lipase differs from most enzyme-systems in that the enzyme is soluble in water while the substrates usually are not. The manner of performing a fat digestion, therefore, is generally admitted to be of unusual importance. By means of a new technic a number of substances, hitherto untested or regarded as unaffected by lipase, have been rapidly split. It cannot be taken for granted that the entire scope of lipase activity is thus displayed, for the methods may still be imperfect. Nevertheless, an examination of these new data leads to the interesting assumption

that the enzyme is exclusively a primary alcohol esterase. The conflict of this statement with the known digestion of triglycerides must be reconciled before it is acceptable. A study of the digestion of synthetic addition compounds of mono- and di-glycerides has so far fitted in with the explanation that the glyceride molecule undergoes rearrangement during hydrolysis. The latest developments of this study, which is still in progress, will be reported.

G15. The Proteolytic Enzymes of Microörganisms. MARVIN J. JOHNSON, University of Wisconsin, Madison.

Recent investigations from several laboratories have added considerably to existing knowledge of the proteinases and peptidases of microorganisms. Although yeasts and a few other microörganisms have been shown to produce proteinases of the papainase type, a large number of microörganisms produce proteinases, characterized by optimal activity at pH 7 and by lack of activation by reducing agents, which do not appear to belong to any previously studied proteinase group. Many bacteria produce a proteinase which splits gelatin readily, but attacks other proteins very slowly if at all. In some species, this is the only proteinase secreted.

The peptidase systems of molds are fairly similar to each other and, in some respects, to previously studied yeast and animal peptidase systems. Bacteria exhibit unusually wide generic differences in their peptidase systems. Examples may be found among the bacteria of almost any known type of peptidase system. Moreover, a number of previously unknown types of peptidases have been found in bacterial cells. Many anaerobic species produce peptidases which are activated by heavy metals and by reducing agents. A number of lactic acid bacteria contain peptidases unique in acting most rapidly in acid media. Some bacterial peptidases are able to hydrolyze unnatural optical forms of peptides, not attacked by previously studied peptidases.

G16. Studies of Respiratory Enzymes Applied to Problems of Bacterial Metabolism. P. W. WILSON, University of Wisconsin, Madison.

The technics originally developed for the study of the respiratory activities of tissue cultures have been modified for the study of bacterial reactions. These technics consist essentially in the use of non-proliferating organisms ("resting" cells) and the measure of enzymic reactions through microprocedures, *i.e.*, the use of microrespirometers, Thunberg

tubes, and microchemical determinations. The chief advantages of this approach to the study of bacterial metabolism are: (1) respiratory activities are separate from those of growth; (2) "isolation" of the enzyme-systems is accomplished through the use of specific substrates and specific inhibitors; (3) data concerned with the kinetics of reaction are readily obtainable; and (4) the experiments are of brief duration, thereby eliminating differences in metabolism associated with cells in various stages of development and allowing comparison of these to be made.

Problems in bacterial metabolism which will be discussed as illustrations of the application of the use of the respiratory-enzyme technics will include: (a) the hydrogen enzymes of *Escherichia coli*—the evidence will be reviewed for the existence of three specific enzymes which are essential for the transfer and liberation of hydrogen by cultures of *Escherichia coli;* (b) the mechanism by which anaerobes secure their energy—discussion will be given of the so-called "Stickland reaction' whereby anaerobes secure energy through reactions involving two amino acids; and (c) species and strain variation among the root-nodule bacteria—a study of the characteristics of the respiratory-enzyme systems of members of the several cross-inoculation groups of the root-nodule organisms will be summarized.

G17. The Rôle of Bacterial Enzymes in Immunology. M. G. Sevag, University of Pennsylvania School of Medicine, Philadelphia.

The phenomenon of catalysis is universal and fundamental in every living cell. It has a direct bearing on the nature and extent of immunological events. The chemical constitutions and specific activities of bacterial enzymes and antigens possess many similar characteristics. The metabolic activity of bacteria is lost as a result of inhibition, impairment or aging. Under these conditions, and in the absence of substrates, organisms begin to undergo autolysis in association with endocellular oxidative processes. Also shown is the increase of oxygen consumption during the lytic action of specific bacteriophage on bacteria. In metabolic studies *in vitro*, the normal course of bacterial enzymic activity can be completely inhibited or modified by the presence of activating or inhibiting agents. These effects under conditions of growth cause structural variations in bacteria. Similar studies on bacteria combined with specific antibody, in the absence or presence of complement, have shown altered metabolic activities. Such influences exercised repeatedly on bacteria cause fundamental changes and alter immunological behavior. Bacterial enzymes are definitely affected by

such phenomena as: agglutination, bacteriolytic complement fixation, bacterial variation in the presence or absence of immune bodies, and phagocytosis in the presence of opsonins and non-specific growth- and enzyme-inhibiting agents.

G18. Commercial Production and Industrial Application of Enzymic Preparations Derived from Microörganisms. LEO WALLERSTEIN, Wallerstein Laboratories, New York City.

The pioneering work of Takamine, Boidin, Effront and others, has enabled considerable progress to be made in the commercial production of highly potent enzymic preparations from bacteria, yeasts and molds. A description will be given of present methods for the manufacture of such preparations and certain of their industrial applications will be discussed.

Historically, the use of enzymic preparations in industry falls into several stages. At first, the effects were simply those of the enzymes secreted by the various microörganisms which happened to be present. Later, the conditions necessary for the desired results were established empirically. With the realization that the reactions were due to certain enzymes and with advancing knowledge of their specific properties, the production of the enzymes themselves naturally followed as a means for controlling the reactions.

The principles involved in the various methods of commercial manufacture will be referred to. As typical, the paper will discuss the production of invertase from yeast, of amylase and protease from *Bacillus subtilis*, and of amylases from *Aspergillus oryzae*. The characteristics of these enzymes and the equipment necessary for their large scale production will be considered. Finally, some important industrial applications will be presented of the ways in which these enzymic products are used in the textile industry, the tanning industry and the various food industries. It is suggested that there are many possibilities for profitable industrial applications of these and other enzymic preparations in new fields. Such applications await only a more general knowledge of the extent to which enzymic preparations are commercially available.

G19. Adaptive Bacterial Enzymes: The Mechanism of Their Production and Their Application in Biochemistry and Immunology. RENÉ J. DUBOS, Hospital of the Rockefeller Institute for Medical Research, New York City.

Karström has classified bacterial enzymes as "constitutive" or "adap-

tive". The former are always produced irrespective of the composition of the culture medium, the latter appear only when the enzyme-substrate is a component of the culture medium. Different theories have been formulated to account for the production of adaptive enzymes: (a) natural selection—this theory assuming that the bacterial species under consideration always gives rise to a few cells (variants or mutants) endowed with the property of producing the specific enzyme, and that these cells are selectively favored when the proper substrate is present in the medium; (b) chemical adaptation—the production of the enzyme being due to a specific chemical interaction between the protoplasm of the cell and the substrate.

Adaptive enzymes of bacterial origin appear to exhibit a remarkable specificity. They can be used, therefore, for the investigation of certain biological problems. Two examples will be discussed: (1) the detection and estimation of creatinine in body fluids by an enzymic method; and (2) the study of the rôle of capsular polysaccharides in determining the specificity and virulence of encapsulated pneumococci.

G20. The Variability of Bacteria with Regard to Their Production of Enzymes. E. J. ORDAL, University of Washington, Seattle.

The subject of bacterial variation with respect to the production of enzymes will be reviewed. There exist conflicting opinions as to whether the bacterial cell can change its enzymic action by producing entirely new enzymes or by discontinuing the production of others. The evidence for the acquisition of new enzymes by bacteria, as well as for the loss of enzymes normally present, will be discussed.

The mechanism of gas production by *Escherichia coli* has been studied by a comparison of the enzymes of normal *Escherichia coli* with the enzymes of anaerogenic variants. The opinion has been expressed in recent years that hydrogen can be produced directly from glucose by means of an enzyme, "glucose hydrogenlyase", which is considered to be separate and distinct from "formic hydrogenlyase", the enzyme producing gas from formic acid. Furthermore, "formic hydrogenlyase" is considered to be separate and distinct from "formic dehydrogenase", the enzyme concerned in the activation of formic acid, and from "hydrogenase", the enzyme concerned in the activation of molecular hydrogen. The evidence obtained from this study indicates that the hydrogen gas produced from glucose by *Escherichia coli* comes from intermediately formed formic acid, and that formic dehydrogenase and hydrogenase are constituents of the enzyme liberating gas from formic acid.

G21. Variation in Certain Biochemical Characteristics of Bacteria. W. J. NUNGESTER, University of Michigan, Ann Arbor.

Variations in certain biochemical activities of an organism of the paracolon type have been studied. This organism did not produce acid from lactose, maltose or sucrose, when grown on a solid medium. After repeated subculturing in liquid media containing these sugars, the organisms changed so as to produce acid readily when grown on agar containing the same sugar. Certain factors were studied with respect to their ability to influence this change in biochemical activity.

It was observed that a decreased oxygen tension, as found in the lower portion of a broth culture or on a solid medium under anaerobic conditions, tended to stimulate the production of acid from the carbohydrate. Dilution of the broth base had the same effect at times. Previous training of the organism to ferment lactose or maltose did not appear to shorten the time required to train it to utilize sucrose. The ability of this organism to ferment the carbohydrates in question was not retained indefinitely. The changes in fermentative action were all of a gradual nature.

G22. Physiological Youth of Bacteria as an Important Factor in Their Adaptive Formation of Enzymes. C. P. HEGARTY, Cornell University, Ithaca, N. Y.

Tryptone broth containing a single sugar was inoculated with 10 per cent of a fully grown culture of *Streptococcus lactis* grown in the same medium. Each hour, samples were centrifuged, the sedimented cells resuspended in phosphate-buffer solutions, and the rate of fermentation of various sugars was determined by titrations at frequent intervals. All of the cells fermented glucose at once at maximal rates. A constitutive enzyme-complex forms lactic acid from glucose.

Glucose-grown cells, one hour old, showed a delay of one-half hour before acid production from sucrose became measurable. Invertase is evidently an adaptive enzyme in this organism. Two- and three-hour-old cells produced acid immediately, and the growth curve showed that these cells were just coming out of the lag phase. Logarithmic growth was established after four hours, and the delay of sucrose fermentation was one hour. Older cells required longer initiation times; eight-hour-old cells requiring three and one-half hours. Plate counts and direct Petroff-Hausser counts showed no increases in the numbers of resuspended cells during fermentation.

Essentially the same results were obtained by growing the cells on

certain other sugars. The time required for adaptation varied both with the sugar on which the cells were grown and the sugar fermented. In each case the physiologically young cells, just coming out of the lag phase, adapted themselves to new carbohydrates much more rapidly than cells in the logarithmic phase of growth.

G23. A Comparison of the Biological Activities of Certain Slow Lactose-Fermenting Bacteria and of Rapidly Fermenting Strains Derived from Them. E. R. HITCHNER, E. A. DONAGAN AND S. ALPERT, University of Maine, Orono.

A comparison has been made of the morphological, cultural, biochemical, and serological characteristics of 15 strains of coliform bacteria, which exhibited delayed lactose utilization, and of rapidly fermenting strains derived from each of them. Most of the strains, which were obtained from several investigators, were isolated from abnormal stools. The rapidly fermenting variants were obtained by several serial passages through lactose broth. Two strains failed to produce rapidly fermenting variants by these methods.

On the basis of their biochemical and serological properties, the organisms were divided into 9 groups. No major differences in the morphological, cultural, and serological characteristics were observed between the slow lactose-fermenting strains and the rapidly fermenting strains derived from each. Five strains which fermented lactose slowly (6 to 9 days) likewise showed a delayed utilization of melibiose. However, coincident with the rapid utilization of lactose, each of these strains acquired the property of fermenting melibiose in 1 day. The rapid lactose-fermenting strains derived from 4 of the cultures fermented raffinose in 1 day, whereas the slow lactose-fermenting parent strains failed to utilize this carbohydrate in 30 days.

These data support the observations of other investigators that microorganisms whose fermentative activity in the rate of utilization of a certain carbohydrate has been markedly accelerated, through suitable "training", will frequently exhibit a similar acceleration in the fermentation of other carbohydrates, toward which they were originally either slightly or completely inactive.

G24. The Sensitivity of Propionibacterium pentosaceum to Sodium Fluoride as a Function of the Conditions of Growth. W. P. WIGGERT AND C. H. WERKMAN, Iowa State College, Ames.

It has been found possible to obtain two types of cells of *Propioni-*

bacterium pentosaceum with respect to sodium fluoride sensitivity. At slightly acid reactions, the glucose dissimilation by cells grown in the absence of sodium fluoride is completely inhibited by this poison, while the dissimilation by cells grown in the presence of the fluoride is not affected. The fermentation of phosphoglyceric acid, a proposed intermediate isolated from a glucose fermentation and inhibited in its breakdown by sodium fluoride, is greater with the former type than with the latter. At higher hydrogen ion concentrations, the cells grown in sodium fluoride are sensitive to this poison.

The phenomena observed may be explained by enzymic differences between the two types of cells, differences in accessibility of fluoride and substrate to the enzymes, or possibly by differences in both accessibility and enzymic character. Evidence for each possibility will be considered and the implications from the standpoint of intermediate metabolism will be discussed.

G25. Dissimilation of Citric Acid by Streptococcus paracitrovorus. C. R. Brewer and C. H. Werkman, Iowa Agricultural Experiment Station, Ames.

The dissimilation of citric acid by *Streptococcus paracitrovorus* was investigated by a chemical analysis of the products and by the Warburg respirometer technic. The organism does not readily attack citrate in a yeast-extract medium, but actively ferments glucose in a similar medium. When citrate and glucose are present together, the fermentation is enhanced and the citrate is rapidly dissimilated.

The presence of small amounts of sugar catalyses the breakdown of citrate, both aerobically and anaerobically. The catalytic effect is not due to the activity of the Krebs citric cycle, because it proceeds anaerobically and is insensitive to arsenite. The addition to citrate of acetylmethylcarbinol or fumarate as hydrogen-transfer agents does not produce stimulation comparable to the addition of glucose.

Streptococcus paracitrovorus does not exhibit oxygen consumption during aerobic dissimilation of citrate, glucose, or citrate plus glucose, as measured by manometric methods. Aerobic dissimilation is insensitive to arsenite.

G26. Enzyme-Systems in Nodules of Leguminous Plants. D. W. Thorne and Robert H. Burris, University of Wisconsin, Madison.

A technic has been developed for the study of fresh tissue from legume-root-nodules. After separating the enclosed bacteria from the

remainder of the nodule tissue, it was found that the enzymic activity of the organisms greatly exceeded that of the surrounding material.

A comparison of the activity of the enzyme-systems of nodular material with that of non-proliferating cells of related species of *Rhizobium* showed a similar action on several substrates including glucose, arabinose, sodium fumarate, sodium succinate and sodium pyruvate. The Q_{O_2} values were considerably greater, however, in the case of the artificially cultured bacterial suspensions. The bacterial suspensions appeared to be more sensitive to some inhibitors than were the related nodular materials. Such differences were particularly evident with respect to fluoride and, to a somewhat lesser degree, to cyanide. In no instance was there any apparent sensitivity to carbon monoxide. The present status of this investigation indicates that the enzymic activities of nodules of various leguminous plants may differ considerably.

G27. Respiratory Enzymes of the Root-Nodule Bacteria. ROBERT H. BURRIS AND D. W. THORNE, University of Wisconsin, Madison.

Good and poor strains of *Rhizobium* from four cross-inoculation groups have been tested for the response of their respiratory activity to changes in pH, temperature and pressure of oxygen. The effect of a variety of inhibitors has been studied. On the whole the bacteria have been very similar, the only marked differences being that the soybean organisms have a higher pH optimum than the other species, and that the clover organisms require a higher oxygen tension than the alfalfa and soybean organisms for maximal respiration.

G28. The Production of Rennin by Pathogenic Streptococci. COSTANTINO GORINI, Milan, Italy.

In 1932, I proposed the milk-on-agar-culture method for detecting the production of rennin by microbes (*Schizomycetes, Actinomycetaceae* and *Saccharomycetaceae*) which fail to show it when inoculated directly into milk (*Bacillus typhi, Bacillus morgani, Bacillus lipolyticum, Streptococcus equi, Streptococcus equinus, Actinomyces aureus, Actinomyces asteroides, Saccharomyces cerevisiae, Saccharomyces lactis*, etc.). The method is described in the "Manual of Methods for Pure Culture Study of Bacteria".

The method has been applied to 168 strains of streptococci derived from pathologic sources. In ordinary milk cultures, these organisms were considered to be negative or quite irregular in respect to the pro-

duction of rennin. Among the strains, 36 were *Streptococcus equi;* 8, *Streptococcus equinus;* 39, *Streptococcus mastitidis;* 9, *Streptococcus scarlatinae;* 7, *Streptococcus epidemicus;* 5, *Streptococcus cardioarthritidis;* 2, *Streptococcus morbilli;* 2, *Streptococcus viridans;* 1, *Streptococcus erysipelatis;* 1, *Streptococcus hemolyticus;* most of the others were pyogenic streptococci. All of these strains regularly curdled the milk-on-agar cultures, almost without the appearance of acid.

Errors in this rennin test arise from at least three sources: (1) the use of unsuitable milk; (2) failure to observe the results at the proper time; and (3) failure to recognize a minute coagulum. If this test be properly carried out and interpreted, it is useful for recognizing pathogenic streptococci in conjunction with other suitable procedures.

G29. A Comparison of Anaerobic Methods. NORMAN J. MILLER, ORVILLE W. GARRETT AND PAUL S. PRICKETT, Mead Johnson and Co., Evansville, Ind.

Many procedures have been proposed for the cultivation of anaerobic bacteria. Some are complicated or require expensive equipment. Simple methods are needed not only for isolation and qualitative studies, but also for quantitative work, especially in laboratories limited in equipment and resources. Three of the simplest methods described in the literature have been compared with each other and with a modification of another previously described simple technic. One of the first three methods was eliminated because of the breakage of glassware encountered. Thus, the three methods finally compared were: Weiss and Spaulding's, Spray's, and a modified deep agar shake. This last procedure, although long recognized as valuable, has been restricted largely to isolative and qualitative technics, doubtless as a result of difficulty in making accurate counts in a round tube.

The use of an oval test tube, the two broader sides of which are approximately parallel, enabled us to develop a more accurate deep agar shake method suitable for quantitative studies. As purchased, the oval test tube is difficult to plug, the agar has a tendency to wet the plug on agitation following inoculation, and a large surface is exposed for the absorption of oxygen. To overcome these drawbacks, we secured oval test tubes that have a round lip and neck. Furthermore, following ZoBell, we cover the inoculated agar with a plug of glucose-peptone-methylene blue agar to serve as an oxygen seal and as an indicator of anaerobiosis. In our hands Weiss and Spaulding's method has proven to be the most accurate and satisfactory, with the modified deep agar shake a close second.

G30. A Simple Technic for Concentrating Tubercle Bacilli in Sputum. N. P. SULLIVAN AND H. J. SEARS, University of Oregon Medical School, Portland.

The method consists in adding to sputum a small quantity of the enzyme papain in powdered form, and incubating the mixture for a few minutes. No further treatment is necessary before centrifuging. The concentration achieved is equal to that obtained by commonly used technics. The disintegration of the mucoid material is rapid and complete, and the sediment obtained spreads easily and adheres well to the slide. The bacteria and other cellular material are unaffected by the process. Cultivation may be obtained by treatment of the sediment by the usual methods.

G31. The Evaluation of a Group of Germicides by the Tissue-Culture Technic. A. J. SALLE, University of California, Berkeley.

It is customary to rate disinfectants on the basis of their phenol coefficients. This may be justifiable when the germicides are to be employed on external skin surfaces or for the sterilization of non-living material. However, when the disinfectants are administered internally or are used on mucous surfaces, the method has serious drawbacks. It is believed that a more accurate method of rating germicides for clinical use can be achieved by testing them for their effect on the growth of living tissue as well as for their ability to kill bacteria. A number, known as the "toxicity index", has been determined. It may be defined as the ratio of the highest dilution of disinfectant showing no growth of tissue in 10 minutes to the highest dilution required to kill the test-organism in the same period of time. Theoretically, the smaller the index the more nearly perfect the germicide.

The toxicity indices of a number of compounds were as follows when *Staphylococcus aureus* was used as the test-organism: iodine, 0.2; Hexylresorcinol, 0.9; silver lactate, 0.9; *p*-hydroxyphenyl-n-amyl sulfide, 1.0; silver citrate, 1.0; *o*-n-hexyl-phenol, 1.1; *p*-hydroxydiphenyl sulfide, 1.3; Metaphen, 1.5; silver protein strong, U.S.P., 1.7; silver nitrate, 1.8; phenol, 2.0; silver protein mild, U.S.P., 2.5+; Mercurochrome, 7.2+; Merthiolate, 169+.

When *Eberthella typhosa* was used, the following were the values obtained: silver nitrate, 0.11; silver citrate, 0.14; silver protein strong, U.S.P., 0.14; silver lactate, 0.15; iodine, 0.2; Metaphen, 0.4; silver protein mild, U.S.P., 0.43; Mercurochrome, 0.6; Hexylresorcinol, 0.8; *p*-hydroxydiphenyl sulfide, 1.0; *p*-hydroxyphenyl-n-amyl sulfide, 1.0; *o*-n-hexyl-phenol, 1.1; phenol, 1.2; Merthiolate, 1.6.

G32. A Method for the Bacteriologic Testing of Chemical Solutions Used for the "Cold Sterilization" of Surgical Instruments. GEORGE F. REDDISH AND ELLA M. BURLINGAME, Lambert Pharmacal Co., St. Louis, Mo.

The present method of the Food and Drug Administration for testing "Preparations for Surgical Instruments" is satisfactory for ascertaining the disinfecting power of such solutions, but not for determining complete sterilization. There is need for a standardized procedure for testing such preparations for their sterilizing ability.

The average number of aerobic spores found on surgical, dental, and veterinary instruments after use in practice, and on contaminated razor blades, was found to be 2, the maximal number found on any instrument being 9. Based on these findings, the following method is suggested: Spread on Gillette-type razor blades 100 times the maximal number of spores found on contaminated instruments (approximately 1000), using spores of *Bacillus anthracis* from a five-day culture grown at 20°C. on nutrient agar of the following composition: 1 per cent Armour's peptone, 0.5 per cent Liebig's beef extract (Lemco), 0.5 per cent NaCl in distilled water, adjusted to pH 7.4. Allow to dry at 37°C. for 1 hour and then expose to the action of the solution being tested for 10, 20, and 30 minutes at 20°C. At the end of these periods, wash the blades in 20 cc. of sterile water for 1 minute and then transfer to 20 cc. of sterile broth of the above composition, adjusted to pH 6.8. Incubate for 3 days at 37°C. A satisfactory sterilizing solution should kill the spores of *Bacillus anthracis* under these conditions within 20 minutes. Practical tests with sterilizing solutions of known merit have shown this method to be satisfactory.

G33. Verification of the Results Secured by the Manometric Method of Evaluation of Germicides. J. BRONFENBRENNER, A. D. HERSHEY AND J. A. DOUBLY, Washington University School of Medicine, St. Louis, Mo.

Recently we suggested a method for evaluation of chemotherapeutic agents based on the estimation of the depressant effect on the rate of oxygen consumption by bacteria and by tissue suspensions, respectively, in glucose-succinate buffer. The present studies were undertaken with a view of correlating the data secured with those obtained by the usual methods for evaluation of germicides and by the animal test for evaluation of the toxic properties of a few representative disinfectants, phenol, Hexylresorcinol, iodine and 4 mercurial compounds.

We found that the difference in the intensity of depressing action of

different germicides was only slightly greater than the possible error due to variation in the manometric measurements themselves, which may involve an error of ± 5 per cent. The time interval chosen for the final reading was of little importance, since practically the full effect of the disinfectants was manifested within 5 or 10 minutes of exposure and little change in the oxygen uptake took place beyond that time.

The analogous values (± 90 per cent of inhibition) for the effect of disinfectants on liver cells were taken as a measure of toxicity and were verified by the experiments *in vivo*. Obviously, direct comparison between the dilutions effective *in vivo* and *in vitro* would be meaningless, since the findings *in vivo* vary according to species, age of animals, route of injection of germicide, etc. Compared on the basis of relative toxicity, however, the results obtained showed remarkably good agreements in all cases except in that of phenol.

G34. A Multiple-Compartment Petri Dish. HAROLD LEON FRUITMAN, San Francisco Water Department Laboratory, San Francisco, Calif.

In the bacteriological examination of 18,000 standard water samples consisting of 5 tubes each, 36 per cent required streaking for confirmation, entailing much labor, a large stock of dishes, and considerable media. Overgrowth by spreaders and diffusion of metabolic products (metallic sheen) were often encountered when several tubes were streaked upon the same plate. Wax markings became illegible and labels were a nuisance. Accordingly, a multiple-compartment Petri dish was designed from Pyrex glass.

The new dish is separated into 5 compartments by rounded baffles (radial spokes) which prevent spreading, diffusion, and media skidding. Maximal streaking surface and ease of cleaning are obtained by the use of sloping floors and rounded fillets. The same amount of time is required to pour agar plates with the new as with the ordinary Petri dish with which it is interchangeable. The outer lower circumference of the dish is sand-blasted for penciled history. An annular ring limits the level of the media to 3 mm. which provides a firm streaking surface and requires but 10 cc. of media for the entire plate. On the bottom are impressed clockwise indices. On the rim are coördinated markings. The first compartment has a quick reference point.

The multiple-compartment Petri dish has many practical uses. It saves media and aids in developing a good streaking technic in classwork. During epidemics, the number, bulk, and expense of Petri dishes and media can be curtailed considerably.

G35. The Advantages of Tungsten Wire for Streaking-Needles. HAROLD LEON FRUITMAN, San Francisco Water Department Laboratory, San Francisco, Calif.

That tungsten wire is the most suitable material from which to make streaking-needles is the conclusion of a four-year study. It possesses the desired qualities of high heat-conductivity and transfer, maximal resistance to oxidation and fatigue, and proper rigidity and elasticity. Over 30,000 tubes during the period of four years have been streaked with a tungsten wire which is only just beginning to show fatigue.

C.P., cleaned and annealed, tungsten wire (General Electric Co.) 22 gauge, is cut to desired size, and held lengthwise in the hot part of a wing-tip flame (600°–1100°C.) until incandescent, then quickly plunged into a saturated solution of sodium chloride; a yellow oxide forms. The wire is repeatedly heated to incandescence until all the oxide and rough flakes are burned off, then plunged into salt solution. The wire takes on a permanently shiny black surface. The wire can be bent into shape either before or after processing, but it should be bent in slow stages while hot.

To streak a set of 5 tubes with a wire made of tungsten required 57 seconds; of platinum, 111 seconds; of nichrome, stainless steel, and chromel, 108 seconds. To become incandescent, a wire of tungsten required 1.4 seconds; of nichrome, 2 seconds; of platinum, stainless steel, and chromel, 3 seconds. When wires were flamed for 3 seconds (wing top used), the cooling times (no steam or sizzling when thrust into water at 30°C.) were: tungsten, 6 seconds; platinum, 17 seconds; nichrome and chromel, 14 seconds; stainless steel, 12 seconds.

G36. A New Type of Needle-Holder. HAROLD LEON FRUITMAN, San Francisco Water Department Laboratory, San Francisco, Calif.

Anyone who does much streaking has noticed a good deal of fatigue of the hand. There has been little change in the design of holders for streaking-needles since the inception of bacteriology. Accordingly, a new instrument has been designed. It is made of aluminum or its alloys, which were chosen after careful consideration of heat conductivity and weight. The design is functional. The needle-holder is as comfortable in use as a favorite pen; it weighs but 20 gm.

The holder is constructed as follows. A rod, 3/8″ in diameter and 7″ in length, is tapered gradually from the nose to a diameter of 1/8″ at the rear tip. A 5/32″ hole is drilled into the rod for half its length. The nose is threaded to receive a two-piece hollow clamp, which consists

of a four-prong interlocking jaw-piece and a circular contractor nut. The jaw-piece is hollow to allow for an adjustable needle length, and the rear end is slotted to facilitate the removal of the clamp. The holder is machined at 1″ intervals to provide a series of encircling concave finger grips. This allows 4 positions, from a close tight pencil hold to a sketch-brush position. Each position has 3 individual grooves (for thumb and fingers) that are ribbed or knurled. Thus, a firm grip is afforded with a minimum of effort. The rear tip is handy for plugging cotton.

G37. Observations on Supersonic Vibration. Paul J. Beard and W. F Gantvoort, Stanford University, Calif.

A device for cooling suspensions undergoing supersonic vibration is described. A possible explanation of the heating effect is advanced. Preliminary experiments indicate that the killing time is related to the volume being vibrated, to the numbers of organisms per unit of volume, to the shape and to the size of the organisms.

Rods were killed more quickly than cocci, large rods more quickly than small rods. All of the organisms in a 30 cc. suspension of *Escherichia coli* containing 3 million organisms per cc. were killed in 40 minutes, while a suspension containing 4.5 million per cc. in a volume of 15 cc. required only 20 minutes for complete killing. A 30 cc. suspension with a density of 20 million per cc. showed 4,000 survivors per cc. after 80 minutes.

G38. Variation of Diphtheria Bacilli: Correlation of Certain Properties with Colonial Form. Harry E. Morton, University of Pennsylvania School of Medicine, Philadelphia.

Six years after the original description of the diphtheria bacillus, variations in the manner of growth were reported. Thus, numerous authors, while working with a variety of strains under different conditions, have recorded many types of variation. These findings must be inventoried, if successful attempts to correlate certain properties with colonial form are to be made, or if an orderly trend within the species is to be disclosed.

Laboratory and freshly isolated strains which have been studied by the author yielded smooth (S), intermediate (SR), rough (R) and dwarf (D) colonial forms. The different forms were isolated directly from clinical sources and occurred spontaneously in cultures. They were also produced by aging, by changes in the pH of the medium and by

the addition of LiCl. The identity of the variants was carefully checked by means of tinctorial, biochemical and immunological reactions. Transformation of a given strain from one colonial type to another was usually accompanied by a change in the degree to which a given reaction was produced, rather than by the loss of certain reactions or the acquisition of new ones. A study of the colonial types showed that what heretofore have been described as discrepancies in the behavior of the diphtheria bacillus are really the normal variations within the species.

G39. A New Bacterial Species Isolated from the Chuckawalla (Sauromalus varius). L. F. CONTI AND JAMES H. CROWLEY, Zoological Research Hospital, San Diego, Calif.

Twenty-five chuckawallas (*Sauromalus varius*), originating on the San Estaban Islands, Gulf of California, were received by the San Diego Zoological Society on April 7, 1937. Tumor-like growths in the inferior cervical regions were noted in the majority of the specimens.

The surgical removal of one well-defined growth followed by bacteriological studies revealed a definite organism in pure culture. This organism differs in several respects from *Serratia anolium* which was isolated and described by F. Duran-Reynals and H. J. Clausen from similar lesions in another species of reptile.

The organism which we found associated with tumor-like growths in chuckawallas is a pleomorphic, Gram-negative bacterium, which actively liquefies gelatin, produces ammonia, reduces nitrates, coagulates and peptonizes milk, and produces a definite yellowish-green fluorescent pigment giving a faint diffuse coloration to the medium. It is motile by peritrichous flagella. Several sugars are attacked with the production of acid and sometimes of gas.

The bacterium has been found in pure culture in every tumor-like growth of living or dead chuckawallas of this species studied by our staff.

The organism was pathogenic to certain cold-blooded animals, but in moderate doses was non-pathogenic to guinea pigs. A soluble toxin was produced. No evidence has been found that the disease is fatal in nature.

Pigment-production studies may permit this organism to be classified in the family *Bacteriaceae*, tribe *Chromobacterieae*, genus *Bacterium*. Hence, this organism is described as a new species: *Bacterium sauromali*.

MEDICAL BACTERIOLOGY, IMMUNOLOGY AND COMPARATIVE PATHOLOGY

M1. The Soluble Malarial Antigen in the Serum of Monkeys Infected with Plasmodium knowlesi. MONROE D. EATON, International Health Division Laboratories, Rockefeller Foundation, New York City.

A soluble antigen is found in the serum of monkeys during the acute stages of the malarial infection caused by *Plasmodium knowlesi*. The antigen disappears from the serum after the acute infection subsides, and sometimes reappears during relapses. It gives specific complement fixation with immune monkey sera and, when injected intravenously into normal monkeys, causes the production of complement-fixing antibodies for malarial parasites without producing infection. Monkeys immunized with serum antigen are not refractory to malarial infection and possess a degree of relative immunity considerably less than that of animals with chronic infection.

The presence of the antigen in the serum is not attributable to extraction of the parasites *in vitro* after collection of the blood, nor to the fragmentation of parasites in the circulation. The antigen is regularly present regardless of the method of obtaining serum. It is not sedimented by ordinary centrifugation and is only partially thrown down at 27,000 r.p.m. for 2 hours. After fractionation with ammonium sulfate, most of the antigen is found in the albumin fraction of the serum, but a part is apparently adsorbed to the globulin precipitable by carbon dioxide. It is labile to heat (70°C.), acids, and alkalis.

An antigen similar to that found in the serum can be extracted by saline from ground parasitized red blood-cells. Antigens from parasitized cells and from serum show differences in their ability to fix complement with various immune sera, indicating that a second antigen not found in the serum is present in the parasitized red cells.

M2. Immunological Relationships of Polysaccharides of Organisms of the Eberthella-Salmonella Group. T. D. BECKWITH AND H. R. MORGAN, University of California, Los Angeles.

The experiments attempted to determine the immunological relationships of polysaccharides prepared from cultures of *Eberthella typhosa*, *Salmonella paratyphi-A*, *Salmonella paratyphi-B*, *Salmonella paratyphi-C*, *Salmonella aertrycke*, *Salmonella enteritidis*, *Salmonella pullorum* and *Salmonella suipestifer*.

Cultures were transferred on three successive days and were then streaked on 1 per cent glucose nutrient agar. Incubation followed at 20°C. or lower in order to obtain growth of a mucoid character and thus a higher content of polysaccharide. Rabbits were immunized against each organism with antigens of this material. The sera of animals immunized with *Salmonella pullorum* showed no agglutinating nor precipitating activity for the homologous antigens. For the precipitation tests, protein-free polysaccharides were prepared from cultures of each organism by the method of Heidelberger, Kendall and Scherp.

Using the antiserum prepared against each organism, agglutination and precipitation tests were conducted with each of the antigens. The cross-reactions between the various organisms in the agglutination and precipitation tests were in general parallel. A marked immunological relationship was indicated in the case of (1) *Eberthella typhosa*, *Salmonella enteritidis* and *Salmonella pullorum* and (2) *Salmonella paratyphi-B* and *Salmonella aertrycke*. These cross-reactions were consistent with the classification of the *Eberthella-Salmonella* group of organisms based on the somatic antigens of White and Kaufman. It is suggested that these polysaccharides represent at least a portion of the surface antigens of the organisms considered.

M3. Certain Newer Aspects of the Antigenic Composition of Hemolytic Streptococci. STUART MUDD, University of Pennsylvania, Philadelphia.

An immunizing substance has been isolated and identified as the type-specific agglutinogen upon which Griffith's classification of streptococcal types is based. This so-called labile antigen has been shown to be a nucleoprotein, and has been split into protein and nucleic acid fractions, each possessing serologic activity (Sevag, Lackman and Smolens, J. Biol. Chem., in press). Labile antigen, on injection into rabbits, produces antibodies without any injury to the animals detectable by histological examination. These antibodies give precipitation reactions to high titer with labile antigens, but have given agglutination only to comparatively low titer when tested with the whole streptococcus. As prepared now, these antigens are not labile to heat and storage, as originally described. Whether this discrepancy has resulted from the presence of enzymatic or other impurities in the earlier preparations, or from other factors, we do not at present know.

Certain nucleic acids isolated by Sevag from the cellular residue of disintegrated streptococci have precipitated specifically with antisera

within Group A, but not with antisera within other Lancefield groups. Nucleic acids may evidently be considered as a new class of specific, serologically active substances.

M4. Antigenicity of Strepto-Fibrinolysin for Rhesus Monkeys. R. R. MADISON AND W. H. MANWARING, Stanford University, Calif.

It was shown by Van Deventer and confirmed by us that the anti-human fibrinolytic enzyme formed or secreted by *Streptococcus hemolyticus* is not antigenic for rabbits, regardless of the dosage, the route or the number of injections. Since this enzyme does not lyse rabbit fibrin, it seemed advisable to test its antigenicity for monkeys. Seven rhesus monkeys, therefore, were given 3 subcutaneous injections of 2 cc. of streptococcal filtrate (1,500 fibrinolytic units) at intervals of 4 days. Fourteen and 21 days after the last injection, these animals were bled and their antifibrinolytic titers determined by our routine technic. The average titers for this group were 3,200 and 5,000 antifibrinolytic units on the 14th and 21st days, respectively.

We conclude from these preliminary data that streptococcal fibrinolysin causes antibody formation in rhesus monkeys. This observation suggests that the lytic enzyme may exert some definite stimulating or toxic effect upon the monkey tissue. This action is not demonstrable in rabbits. This tissue-specific irritation may be the necessary stimulation to the specific production of antibodies.

M5. An Experimental Study of the Treatment of Pneumococcal Toxemia with a Special Carbon Preparation. GEORGE E. ROCKWELL, 2500 Melrose Avenue, Cincinnati, O.

The intravenous injection of carbon is not new. Since Conklin introduced the procedure, Saint-Jacques and others have used it extensively. Although no highly potent pneumococcal toxin has been made, lobar pneumonia in man presents the clinical manifestations of a profound toxemia. The toxin used in the present animal experiments was made by the method of Parker (anaerobic autolysis) or by that of Dick and Boor. The carbon used was animal charcoal dispersed in a saline-glucose solution. The size, the charge on the surface and the volatile content of the particles was controlled. The preparations were standardized for their ability to absorb pneumococcal toxin.

Mice which had been given lethal doses of the pneumococcal toxin

could be saved by the intravenous administration of 1 mg. of carbon, even though the carbon injection was delayed by as much as 4 hours after the injection of the toxin.

Sixteen cases of pneumonia were treated with intravenous injections of animal charcoal in conjunction with antipneumococcal serum or (when that was not possible) with sulfanilamide. Only one case died—a man over seventy years of age who developed acute cardiac failure. The number of cases of pneumonia in our series is too small for statistical analysis. The good results, however, suggest that charcoal may be a valuable adjunct in the therapy of pneumonia. It should be used in conjunction with antiserum.

M6. The Production of Immunity to Experimental Pneumococcal Infection with an Artificial Antigen. WALTHER F. GOEBEL, Hospital of the Rockefeller Institute for Medical Research, New York City.

Artificial carbohydrate-protein antigens, prepared by combining the diazonium derivatives of the *p*-aminobenzyl glycosides of the disaccharide cellobiose and its uronic acid derivative, cellobiuronic acid, with the globulin of horse serum, give rise in rabbits to antibodies which in each instance are directed specifically toward the carbohydrate radical of the azo-protein antigen.

The sera of rabbits immunized with the conjugated cellobiuronic acid antigen agglutinate Type III pneumococci in high dilution. When mixed with cellobiuronic acid antiserum, the microörganisms show the typical Neufeld "quellung" phenomenon. Rabbits immunized with the artificial antigen develop increased active resistance to dermal infections with Type III pneumococci, and their sera confer a high order of passive immunity on mice against experimental infection with virulent pneumococci, not only with Type III organisms, but with Type II and VIII pneumococci as well. Rabbits immunized with the antigen containing cellobiose, on the other hand, are neither resistant to pneumococcal infection nor do their sera confer passive immunity on mice.

The chemical constitution of the cellobioside and cellobiuronide used in preparing the two antigens is identical, save for the grouping occupying the twelfth position in each derivative. In the disaccharide this grouping is a primary alcohol ($-CH_2OH$), whereas a carboxyl group ($-COOH$) occupies this position in the aldobionic acid. This slight alteration in chemical structure suffices, however, to confer upon the

synthetic antigen containing cellobiuronic acid an important immunochemical property, namely the capacity to incite both active and passive immunity against pneumococcal infections in experimental animals.

M7. The Penetration of Antipneumococcal Serum into the Pneumonic Lesion in Rats Following Serum Therapy. ALICE H. KEMPF AND W. J. NUNGESTER, University of Michigan, Ann Arbor.

As a result of previously reported studies on the distribution of pneumococci in rats with experimental lobar pneumonia, it was felt that antipneumococcal serum, following intravenous injection, either did not reach the organisms present in the lungs or for some reason was ineffective there. One possibility was that the larger vessels or the capillaries were not patent; another, that the capillaries were impermeable to the serum. By the use of thorium dioxide (a radio-opaque substance), India ink and trypan blue, the patency of the arteries, arterioles and capillaries, and the permeability of the capillaries were studied. Various serological tests were performed to determine the presence of antiserum in the pneumonic lesion of rats which had been injected with large amounts of antipneumococcal rabbit serum. Animal-inoculation experiments were also done. In general, it was found impossible to demonstrate antibodies in the pneumonic lesion following the administration of large doses of antiserum.

M8. Immunization of Experimental Animals with a Soluble Antigen Extracted from Avirulent Pneumococci. RENÉ J. DUBOS, Hospital of the Rockefeller Institute for Medical Research, New York City.

Pneumococci killed with acetic acid, then extracted at pH 7.0, release in solution a fraction which is insoluble at acid reactions. The sera of rabbits, immunized intradermally with this fraction prepared from an R strain derived from Type II pneumococci, protect mice against infection with large doses of virulent pneumococci. When the immunizing antigen is used in relatively small amounts, the protective antibodies exhibit type specificity, for they afford protection only to mice inoculated with virulent Type II pneumococci. On the contrary, when larger amounts of antigen are used, the sera also protect mice against pneumococci of other types. This heterologous protection, however, is never as high, nor as constant, as that obtained against pneumococci of the homologous type.

It is evident that the antigen described above differs from the classical

capsular-polysaccharide antigen since (1) it can be prepared from avirulent R cells, (2) it is effective in the rabbit when injected by the intradermal route, and (3) the protective antibodies to which it gives rise do not precipitate the capsular polysaccharide.

M9. The Use of the Skin Test with Type-Specific Polysaccharides in Controlling Serum Dosage in Pneumococcal Pneumonia. COLIN M. MACLEOD, K. GOODNER, P. B. BEESON AND C. L. HOAGLAND, Hospital of the Rockefeller Institute for Medical Research, New York City.

The skin test with the homologous specific polysaccharides in controlling the dosage of serum has been employed as a guide in the treatment of 104 patients with pneumonia due to pneumococci of Types I, II, III, V, VII and VIII. The test has been found applicable to the control of the dosage of antipneumococcal serum whether derived from the horse or the rabbit.

Thirteen patients showed a positive skin test before the administration of specific antibody and at a time when the disease was advancing. In this group of patients the test could not be used as a guide to therapy. Ninety-one patients showed a negative reaction before serum was given. In all but one, the test was used to control the dosage of serum, therapy being stopped upon the development of a positive test.

It is concluded that the skin test is a valuable adjunct in the serum therapy of pneumonia provided adequate criteria are maintained for its interpretation.

M10. Some Properties of the Type-Specific Proteins of Antipneumococcal Sera. KENNETH GOODNER, Hospital of the Rockefeller Institute for Medical Research, New York City.

It now appears that several different serum proteins may have the properties of antibodies; indeed, it seems certain that in some immune sera one antigen may react with more than one variety of antibody protein. Moreover, within any one class of serum proteins, there appear to be antibodies having varying affinities for a single antigen.

An antibody protein having the properties of a euglobulin has been separated from Type I antipneumococcal horse serum. The addition of increasing amounts of acetyl-polysaccharide to this antibody solution gives results in terms of antibody precipitated which do not form a curve such as that obtained with whole serum, but which give a figure of ascending straight lines, three phases being clearly outlined. That

these phases may be related to determinate combining groups of the antigen is shown by the fact that the deacetylated polysaccharide gives a figure showing only two ascending phases.

Simple results are also obtained with whole serum and an antigen containing galacturonic acid. It has been shown that this antigen reacts only with an antibody protein which has the properties of pseudoglobulin.

M11. Precise Evaluation of Therapeutic Antipneumococcal Sera. K. GOODNER, C. M. MACLEOD, P. B. BEESON, AND C. L. HOAGLAND, Hospital of the Rockefeller Institute for Medical Research, New York City.

The generally accepted evaluation of antipneumococcal sera is based on the mouse protection system. This can be carried out rather accurately for any one serum of any one type, provided an arbitrary value be assigned to a reference serum and provided enough mice be used for each test. There are two major objections to this system: (1) there is no reference of potency from serum of one type to another; (2) the test is quite expensive, if it be carried out in such a way as to give precise results.

The solution of the problem appears to be the immuno-chemical precipitation method of Heidelberger, Sia and Kendall, as hereinafter qualified. Each mg. of antibody protein in antipneumococcal rabbit serum (Type I) is equivalent to 185 units. Horse sera fall into two groups. The sera of group A have the value of 85 units per mg. of antibody protein, while those of group B have the value of 135 units per mg. These groups can be readily distinguished by the use of non-specific antigens, such as sulfanilic acid combined to a protein, or by differential precipitation at various temperatures. The sera of group A give a marked reaction with the non-specific antigen. They also form relatively less precipitate at 37°C. than do the sera of group B.

The chemical method has the distinct advantage of the elimination of arbitrary standards, since a quantity of antibody protein is directly referable from serum of one type to another.

M12. The Production and Standardization of Diagnostic Antipneumococcal Sera. HAROLD W. LYALL AND HELEN R. ODELL, New York State Department of Health, Albany.

The provision of diagnostic antipneumococcal sera of proven speci-

ficity and potency constitutes a highly important problem in connection with the extended use of serum therapy in pneumococcal pneumonias. Experience in the standardization of relatively large amounts of rabbit sera of all the recognized types has afforded considerable data of interest. Cross-reactions occur more frequently in some of the typing sera than in others. Such reactions may be present more or less regularly and may or may not be reciprocal. Other unpredictable cross-reactions also occur. Extensive tests in which representative strains of all types are used are, therefore, necessary before a serum is released for distribution. Sera with cross-reacting properties may be rendered specific by absorption. The practicability of this procedure is, however, yet to be determined. The "quellung" titers of monovalent sera and of mixtures of several types of sera necessary to procure satisfactory results require further consideration before a definite standard can be adopted. The increasing number of strains of pneumococci which cannot be placed in recognized groups, some closely related to established types, presents a problem from the standpoint of the classification of strains and the standardization of sera—also in the evaluation of the results of specific serum treatment. The establishment of an arbitrary number of types to be included in a serum mixture or pool is not essential, provided sera of satisfactory potency and those with reciprocal cross-reactions are combined.

M13. Coccidioidomycosis. ERNEST C. DICKSON, Stanford University School of Medicine, San Francisco, Calif.

A review of coccidioidal granuloma from a clinical and industrial point of view will be presented. The story of the primary acute infection, which is found in the San Joaquin Valley and which precedes coccidioidal granuloma, will be related. The close resemblance of the primary disease, as well as of the secondary or terminal coccidioidal granuloma, to tuberculosis will be made clear.

The primary infection is caused by the inhalation of the chlamydospores of the fungus. Characteristic pulmonary changes, which are often diagnosed as tuberculosis by roentgen examination, are of relatively short duration. Erythema nodosum is extremely common. The fungus may be recovered from the sputum. The great majority of victims, who think they have had a bad cold or "flu", recover promptly without complications. There is a specific skin-test antigen which indicates unusual hypersensitivity when the patient has erythema

nodosum. A small number of the patients, whether they have had erythema nodosum or not, develop the highly disabling and highly fatal coccidioidal granuloma.

Inhalation experiments have shown that the chlamydospores can produce a characteristic pathological reaction in the lungs which differs from the typical granulomatous change of coccidioidal granuloma. Experiments have also explained the formation of the typical spherules and endosporulating forms which are seen in infected tissues.

M14. An Endotoxin from Aspergillus fumigatus. ARTHUR T. HENRICI, University of Minnesota, Minneapolis.

Cell sap from a pathogenic strain of *Aspergillus fumigatus* is toxic for rabbits, guinea pigs, mice and chickens, while the broth filtrate is harmless. Pathogenic strains of *Aspergillus flavus* and *Aspergillus oryzae* show a similar but lesser toxicity. The cell sap is hemolytic *in vitro*. Injected subcutaneously in rabbits, it produces a massive gelatinous exudate similar to that resulting from *Clostridium oedematiens* toxin, with central purpura and necrosis. Intravenously and intraperitoneally, large doses cause death within 48 hours, with pulmonary congestion and hemorrhages; with smaller doses death is delayed up to 2 weeks, and fatty changes with necrosis are found in liver and kidneys. An extensive serofibrinous exudate in pleural or peritoneal cavities is common but not constant. Guinea pigs often develop a paralysis, beginning in the hind quarters and becoming general before death; they are usually found dead in a sitting posture. The toxin is not neutralized by sodium ricinoleate, in fact its action is accelerated. In this, as well as in the nervous symptoms and visceral lesions, the toxin closely resembles that of *Amanita phalloides;* it differs, however, in being heat-labile and non-toxic by mouth. It resists 55°C. for 45 minutes, but is inactivated at 62°C. for 15 minutes. Rabbits and guinea pigs may be immunized actively so that they tolerate 10 or more lethal doses; but a long drawn-out immunization is required, and many animals are lost in the process. The serum of actively immunized rabbits affords passive protection to guinea pigs, and neutralizes the hemolytic action *in vitro*.

M15. On the Spirocheticidal Action of Arsenic and Bismuth Compounds in Vitro. HARRY EAGLE, Johns Hopkins Hospital, Baltimore, Md.

Arsphenamine and some of its derivatives (neoarsphenamine and

silver arsphenamine), "arsenoxide", various inorganic arsenicals, and bismuth compounds all immobilize and kill pathogenic *Treponema pallidum in vitro*. Some of these substances have a direct effect on the organism. In the case of others (*e.g.*, neoarsphenamine), the preliminary oxidation by atmospheric oxygen seems to be essential for antispirochetal activity. There are yet other compounds (*e.g.*, sulpharsphenamine) which are almost entirely inert *in vitro*, even in the presence of atmospheric oxygen, which are nevertheless actively therapeutic in human and rabbit syphilis.

M16. Epidemiological Studies on Weil's Disease, with Notes on the Susceptibility of the "Gopher," Citellus richardsonii (Sabine), to the Experimental Inoculation of Leptospira icterohemorrhagiae. JEROME T. SYVERTON, W. W. STILES AND GEORGE PACKER BERRY, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

It has been found that the "gopher" or Richardson ground squirrel, *Citellus richardsonii* (Sabine), is susceptible to the etiological agent of Weil's Disease, *Leptospira icterohemorrhagiae*.

A human and a rat strain of this organism were used to initiate in gophers 2 transmission series which were carried through 15 and 5 successive passages, respectively. Transfers of the organism were effected by the parenteral introduction of blood or kidney-suspension derived from the preceding host. Following the final passage, the organisms were recovered and their unaltered pathogenicity for guinea pigs was demonstrated. Transfers of the infection were also accomplished by permitting normal gophers to feed on their cage mates, which were moribund or dead of experimental Weil's Disease. It is suggested, therefore, that the gopher and similar wild rodents may act in nature as intermediate hosts for the spirochete.

With the aid of this highly susceptible new host, and of a previously described simple laboratory procedure which uses guinea pigs for the detection of strains of *Leptospira icterohemorrhagiae* of low virulence, we have isolated 27 strains of this organism from 170 wild rats and 2 strains from human cases of Weil's Disease. The rats were obtained in Rochester, N. Y., (18 of 47 rats positive), Detroit, Mich., (7 of 42 rats positive), and San Francisco, Calif., (2 of 12 rats positive). Neither human cases of Weil's Disease nor the carrier state in rats has been established previously in Detroit or Rochester.

M17. The Inactivation of Gonococcal "Toxin" in Vitro by Sulfanilamide. C. M. CARPENTER, G. M. BARBOUR AND P. L. HAWLEY, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Recently we have observed that sulfanilamide will protect mice against lethal amounts of "toxin" prepared from a strain of *Neisseria gonorrhoeae* isolated from the knee joint of a patient with gonococcal arthritis. Because of this finding *in vivo*, tests were made to determine whether sulfanilamide had an effect on gonococcal "toxin" *in vitro*. The "toxin" employed was simply a lyophilized and regenerated whole 6-day ascitic fluid broth culture of the above strain of gonococcus. Since we had found that 10 mg. of sulfanilamide were required to protect mice against 0.1 cc. of this material, mixtures of sulfanilamide and "toxin" were prepared in this ratio. The mixtures were used after incubation at 37°C. for 24 hours. Their pH values did not change significantly during incubation. Mice in groups of from 15 to 50 were injected with the sulfanilamide-"toxin" mixtures. Controls were provided by mice in similar groups which received untreated "toxin," *i.e.*, "toxin" unmixed with sulfanilamide, but incubated at 37°C. for 24 hours. Combining the mortalities noted in the various tests, it was found that only 25 per cent of the 295 mice injected with the sulfanilamide-"toxin" mixtures died, while all of the 116 control mice injected with the untreated "toxin" succumbed.

M18. The Cultivation of Neisseria gonorrhoeae on the Chorio-Allantoic Membrane of the Chick Embryo and the Use of This Technic for the Study of Sulfanilamide. GRANT MORROW AND GEORGE PACKER BERRY, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The lack of any animal which is susceptible to infection with *Neisseria gonorrhoeae* has greatly hindered the study of gonococcal infections. Since several types of highly parasitic bacteria have been cultivated on the chorio-allantoic membrane of the chick embryo, it seemed important to ascertain whether this *in vivo* technic would succeed in the case of the gonococcus. Such proved to be so. Using the method of Goodpasture and Buddingh, we have been able to grow both old laboratory and freshly isolated strains of *Neisseria gonorrhoeae*. Each of 2 old and 2 new strains has been readily carried through 25 and 10 egg-transfers, respectively. The characteristics of the organisms have not changed

perceptibly. In the eggs, the infection seems to be localized to the chorio-allantoic membrane; apparently the embryo has not been invaded.

The successful cultivation of gonococci on the chorio-allantoic membrane has enabled us to study *in vivo* the prophylactic and therapeutic effects of sulfanilamide. When 0.3 cc. of a 1.6 per cent solution of the drug were dropped on the chorio-allantoic membrane 24 hours before inoculation with gonococci, no infection occurred. When the drug was used therapeutically after a gonococcal infection had been established for 24 hours, however, the amount of sulfanilamide required to eradicate the organisms was twice the prophylactic dose. These statements are based on a number of experiments with 2 old laboratory strains. It is obvious that this method provides an opportunity for testing other chemotherapeutic agents.

M19. Experimental Staphylococcemia in White Mice Produced by the Intraperitoneal Injection of Mucin-Suspended Staphylococci.
B. S. Levine, National Institute of Health, U. S. Public Health Service, Washington, D. C.

Preliminary to the initiation of studies in chemotherapeusis of staphylococcemias, attempts were made to develop a method for the production under controlled conditions of experimental staphylococcemia in white mice.

Series of dilutions of 5-hour staphylococcal broth cultures were made in saline alone, for control purposes, and in mucin, as described by Miller. Doses of 0.5 cc. of each were injected intraperitoneally into white mice. Mucin increased the susceptibility of the mice, but not to as great a degree as was expected. When the dosage and the animal's susceptibility were favorable, death occurred within 48 hours and, in the absence of contaminant-invasion, was due primarily to the effect of the exostaphylotoxin and secondarily to the staphylococcal invasion. Mice dead as a direct or indirect result of the experimental inoculation were autopsied as soon as possible. Mice surviving for one week were etherized and autopsied at once. Bacteriological studies were made of the heart-blood of all mice.

The rapid death of mice injected with high dilutions of the staphylococcal broth culture in mucin, or death occurring several days after injection with low dilutions, in nearly every instance, was due to the accidental presence of enteric bacteria, either in pure culture or in

dominant admixture. Contamination with bacteria culturally simulating *Eberthella enterica*, *Salmonella icteroides*, and *Salmonella abortivo-equina* proved most fatal to the mucin-injected white mice.

In the surviving mice, the immunological mechanism appeared to be two-fold: (1) serum immunity, with heart-blood sterile, and (2) localization in pure culture of the original staphylococci in abscesses.

M20. Failure of Vitamin C to Potentiate Formed Antigens. R. R. Madison and W. H. Manwaring, Stanford University, Calif.

We have previously shown that the injection of horse serum plus ascorbic acid (or in association with this enzymic activator) gives a ten-fold greater yield of antihorse precipitins in rabbits than that obtained after control injections with non-potentiated horse proteins. Vitamin C, however, does not increase the yield of antibodies against formed elements. Ninety-six rabbits have been injected in groups with such formed antigens as sheep erythrocytes, *Eberthella typhosa*, *Proteus vulgaris*, or *Diplococcus pneumoniae*. Without exception, agglutinin, precipitin and Forssman-antibody formation were identical in the Vitamin C-potentiated rabbits and in the control animals. This observation suggests the tentative conclusion that two different mechanisms for the specific formation of antibodies must be operative in rabbits; one capable of potentiation (or stimulation) by Vitamin C, the other refractory to this enzymic activator.

M21. An Outbreak of Botulism in Captive Mink on a Fur Farm in Colorado. Ivan C. Hall and George W. Stiles, University of Colorado School of Medicine and Hospitals and U. S. Bureau of Animal Industry, Denver.

The death of 146 out of 148 mink on a fur farm near Denver, Colorado, was shown to have been caused by the toxin of *Bacillus botulinus* (Type A). This is believed to be the first recorded instance of naturally occurring botulism in mink or other fur-bearing animals. The financial loss, after salvage of the pelts, was approximately $7,000. The toxin was demonstrated in filtrates of scrapings from the feeding boards, of the stomachs of the dead mink, and of their feces. It proved to be impossible to trace the origin of the toxin. All of the examinations of suspected foods, frozen hog livers, commercially canned fish of two brands and commercially canned tomato purée gave negative results for toxin.

M22. Atypical Gram-Negative Organisms from Cases of Acute Intestinal Disorders. F. E. COLIEN, Creighton University School of Medicine, Omaha, Neb.

Stool specimens from cases of acute intestinal disturbance and autopsy material from one case were examined on modified Endo's and eosin-methylene blue media and on Entogenic Agar (Bacto). Gram-negative short rods more nearly resembling members of the dysentery group than any other, yet differing from any of the recognized members of that group in some biochemical or serological reaction, were isolated from all cases, in some instances in pure culture. The biochemical reactions of these organisms were studied in relation to strains of *Shigella paradysenteriae* (Flexner variety), *Shigella paradysenteriae*, var. *sonnei*, *Shigella dispar*, *Shigella ambigua* (Schmitz bacillus) and *Shigella alkalescens*. The antigenic relationship of these organisms to the strains of dysentery bacilli and to members of the coli group was also investigated. The blood sera from several of the cases agglutinated the organisms described in a dilution of 1:80.

M23. A Study of Strains of Shigella Isolated from Urine. LUTHER THOMPSON, Mayo Clinic, Rochester, Minn.

In a series of 5,000 cultures of urine which were made in 1935, about 0.5 per cent of the cultures contained organisms belonging to the genus *Shigella*. During the period between September, 1937, and March, 1938, 13 strains of *Shigella* were isolated from urine and studied by cultural and serological methods. All belonged to the mannite-fermenting group and were negative in lactose and sucrose. Most strains fermented dulcitol and xylose and produced indol. All strains were nonmotile and failed to liquefy gelatin.

Agglutination tests with sera prepared by immunizing rabbits with 4 separate strains showed that the cultures were antigenically heterogeneous. Unless some contrary evidence can be found, it would seem best to consider these organisms as strains of *Shigella alkalescens* showing minor variations in fermentation. Agglutination tests indicate that there are 2 serological groups which include 7 of the 13 strains.

M24. Viruses and Virus Diseases. THOMAS M. RIVERS, Hospital of the Rockefeller Institute for Medical Research, New York City.

For a long time many workers refused to admit that virus diseases should be placed in a special class and that the viruses themselves should

be looked upon, except for size, as a special group of infectious agents. At present most workers agree that there is a special group of agents different from ordinary protozoa, fungi, bacteria, spirochetes and rickettsiae. However, there is no general agreement regarding the nature of the viruses.

The outstanding work of Stanley, in which a nucleoprotein composed of extremely large molecules and possessed of all the characteristics of the causative agent of tobacco mosaic was obtained in crystalline form from the sap of diseased plants, has struck the imagination of a host of workers. Many of these investigators have rushed to the conclusion that all viruses are macromolecules.

Stanley's findings have been abundantly confirmed, but the origin of the macromolecules and the mode of their reproduction are not known. Some of the explanations are fanciful and one should be slow to accept them without more convincing evidence. Furthermore, there is already sufficient evidence that some of the viruses, *e.g.*, those represented by elementary bodies, are entities of a much greater complexity than are Stanley's macromolecules. Consequently, it is not wise at present to make too many generalizations regarding the nature or mode of reproduction of the agents placed in the virus group, because no evidence has been brought to show that all of them must be identical or similar in nature.

M25. Antigenic Differences in Strains of Epidemic Influenza Virus. THOMAS FRANCIS, JR., AND T. P. MAGILL, International Health Division Laboratories, Rockefeller Foundation, New York City.

In 1937, Magill and Francis reported that 2 strains of epidemic influenza virus which had appeared to be identical immunologically could be differentiated serologically. These studies have been extended to embrace observations on the antigenic constitution of 24 strains of the virus. Two methods have been employed: (1) cross-neutralization tests using the various strains of virus and sera prepared against these strains by the intraperitoneal vaccination of rabbits; (2) cross-active immunity tests in mice immunized by the intraperitoneal inoculation of virus. By the use of these procedures it has been possible to show that distinct differences in strains of epidemic influenza virus are detectable. In some instances the serological differences are quite marked; in other instances, extremely slight. The significance of these variations will be discussed.

M26. A Soluble Antigen from Infectious Myxomatosis in Rabbits. THOMAS M. RIVERS, S. M. WARD AND J. E. SMADEL, Hospital of the Rockefeller Institute for Medical Research, New York City.

The occurrence of a soluble antigen in virus-free filtrates prepared from infected skin and from serum of rabbits sick with myxoma has been previously reported. Results of present studies on the nature of this antigen are summarized in the following manner: it is destroyed by a temperature of 56°C., by hydrogen ion concentrations greater than pH 4.0, or by formaldehyde in concentrations greater than 0.5 per cent; it is a globulin with an isoelectric point close to pH 4.5; it is not appreciably affected by digestion with commercial trypsin; on storage gradual loss of ability to form a precipitate with myxoma-immune serum occurs. Purification of the antigen was attained by the precipitation of the crude preparation at different pH values, with or without ammonium sulfate, followed by tryptic digestion.

Partially purified antigen, which was obtained from serum and which reacted with immune serum in a dilution of 1:10,000, gave no evidence of the presence of nucleic acid, gave a faintly positive Molisch reaction, contained 15.4 per cent of nitrogen, and on spectroscopic examination showed bands typical of the aromatic amino acids present in ordinary globulins. Rabbits immunized with partially purified antigen developed in their serum precipitins against the antigen, but responded as did normal animals to inoculation with the viruses of myxoma, neuromyxoma or fibroma.

M27. A Non-Lethal "Mutant" Strain of Virus myxomatosum Derived from Fibroma Virus. GEORGE PACKER BERRY, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

A non-lethal "mutant" strain of *Virus myxomatosum*, named "80-A", has been obtained by transformation from the virus of rabbit fibroma (Shope). This "80-A" strain came from a rabbit inoculated with a mixture of active fibroma virus (Cayuga strain) and heat-inactivated myxoma virus (Brazilian). The fibroma virus was employed as a 1 per cent suspension of fibromatous testicle, the inactivated myxoma virus as a suspension of washed elementary bodies (titer 10^7) which had been heated at 80°C. for 30 min.—a procedure which is invariably lethal for myxoma virus and which usually inactivates the "transforming agent".

The word "mutant" is used in describing the "80-A" virus, because

the strain appeared suddenly with distinctive characteristics which have persisted for 13 months through 23 passages in rabbits. The most striking feature of "80-A" myxomatosis is the low mortality rate (15 per cent from the uncomplicated infection). In the rabbit, both clinically and pathologically, the condition is easily differentiated from fibroma or ordinary myxomatosis. It also has distinguishing characteristics on the chorio-allantoic membrane of the chick embryo. Among other differences, it has been more difficult to cultivate than myxoma virus. After a few passages in eggs, it has behaved typically on retesting in rabbits. A "transformation experiment" using a heat-inactivated suspension of the elementary bodies of "80-A" virus (titer 10^7; 65°C. for 30 min.) and active fibroma virus (Cayuga) has yielded a modified "myxoma" virus which is apparently identical with "80-A". Its characteristics have persisted for 4 months through 7 passages in rabbits.

M28. A Further Study of the Cultivation of Virus myxomatosum on the Chorio-Allantoic Membrane of the Chick Embryo. RACHEL E. HOFFSTADT AND K. STEPHEN PILCHER, University of Washington, Seattle.

Recently Hoffstadt and Pilcher reported the cultivation of *Virus myxomatosum* on the chorio-allantoic membrane of the chick embryo. Further studies have been made on the factors influencing the growth and reaction of the virus in the embryo. The temperature for optimal development was found to be from 33° to 35°C. The virus, after many serial passages, apparently adapted itself to growth within the egg. The virus produced infection in the embryos, as indicated by gross pathological changes, changes in the blood counts and in a reduction in the percentage of hatch of the eggs.

M29. Effect of Solutions of Sucrose, Glycerol, and Urea on the Size and Density of the Elementary Bodies of Vaccinia. J. E. SMADEL, E. C. PICKELS AND T. SHEDLOVSKY, Rockefeller Institute for Medical Research and International Health Division Laboratories, Rockefeller Foundation, New York City.

The rate of sedimentation of the elementary bodies of vaccinia was studied in the ultra-centrifuge of Bauer and Pickels with the light-absorption method of Svedberg. In dilute buffer solutions with pH values close to 7.0, the single virus particles sediment at a rate of approximately 49×10^{-11} cm./sec./dyne. The rate of sedimentation of the

elementary bodies increases after suspension in solutions of sucrose, glycerol, and urea. Different maximal rates are obtained in these solutions having the same specific gravity. The maximal rate is generally maintained in solutions of sucrose, but in concentrated urea it subsequently declines. The sedimentation rate returns to normal when the elementary bodies are resuspended in dilute buffer solution.

The density of elementary bodies suspended in dilute buffer is estimated as 1.16 gm./cc. However, the density is increased by contact with solutions of sucrose, glycerol, or urea. For example, it becomes 1.25 gm./cc. in a 53 per cent solution of sucrose. With sucrose, the increase in density may be accounted for by an osmotic extraction of water from the particles. On this basis one-third of the volume of the particle is extracted by a solution of sucrose of specific gravity 1.25. The behavior of the elementary body in solutions of urea is complicated by its permeability to urea. The effect of glycerol is intermediate between that of sucrose and urea.

M30. Experiments on Antirabic Immunization. ANSON HOYT, FREDERICK J. MOORE, M. KATHERINE GURLEY AND DOUGLAS WARNER, University of Southern California School of Medicine, Los Angeles.

The sera of goats and rabbits, hyperimmunized with rabies fixed virus, were tested for neutralizing and protective properties. White mice were employed as the test animals, all virus inoculations being given by the intracerebral route. Both goat and rabbit antisera possessed high neutralizing titers against fixed virus. In experiments on protection, the rabbit serum proved greatly superior to goat serum. Rabbit serum injected intraperitoneally before the inoculation of street virus brought about an extremely high percentage of survival, whereas almost all untreated control animals contracted rabies. Rabbit serum also demonstrated considerable protective effects even when administered as late as four days after street virus.

Experiments on combined passive and active immunization against fixed virus showed that a single injection of antirabic rabbit serum, given at the beginning of active immunization, largely nullified the effects of 12 daily doses of vaccine. Mice receiving vaccine alone began to demonstrate active immunity immediately following 5 daily injections. Further vaccination produced an immunity of marked degree. Mice could not be protected against fixed virus, if active immunization were

begun after the inoculation of virus. However, it was possible to protect mice when vaccination was started after the administration of street virus. A possible explanation lies in the fact that fixed virus begins to multiply rapidly in the brains of mice about two days after inoculation, whereas it takes approximately six days for the demonstrable multiplication of street virus.

M31. Plant Viruses as Factors in Morphogenesis. MICHAEL SHAPOVALOV, Bureau of Plant Industry, U. S. Department of Agriculture, Logan, Utah.

Viruses tend to alter host-plant morphology normally controlled by genes and environment. Many of these virus-controlled changes can be transmitted by insects, grafting, or the injection of juice.

The morphogenetic effects of viruses may be largely referred to the sphere of teratology. In their manifestation these effects are either suppressive, as in dwarfing or the filiformity of normally broad-leaf blades, or stimulative, as in excessive vegetative growth, protuberance formation or leaf branching. Infectious leaf variegation is a form of chlorophyl-suppressing activity of the virus. When the suppressing effect of a virus is partially overcome by normal processes of the plant, peculiar manifestations of suppressed recovery result, as in recovery from curly top virus.

Virus-induced malformations often parallel aberrant genetic forms. Thus, "shoestring" tomato mosaic closely resembles "wiry" mutant. Leaf variegation may be caused by either virus or genetic factors, and leaf branching may arise from either genetic or virus causes.

As teratological phenomena, readily obtainable and easily controlled, virus-induced malformations may be of great value in experimental morphology. In some cases they present the best means for revealing the plant's phylogenetic history.

The application of form-building viruses in ornamental horticulture, although rare and limited to certain specific forms, is nevertheless established and has produced such plants as some of the variegated abutilons, tulip Rembrandt, etc. Further possibilities in this respect, particularly with reference to the culture of dwarfed plants, deserves attention.

M32. Studies on Selected Strains of Curly Top Virus. N. J. GIDDINGS, Bureau of Plant Industry, U. S. Department of Agriculture, Riverside, Calif.

Four strains of curly top virus, designated 1, 2, 3, and 4, were recog-

nized by the differential reactions of varieties of sugar beet (*Beta vulgaris* L.). Percentages of plants infected and severity of symptoms were used as bases of comparison. Strains 1 and 3 induced severe symptoms in susceptible beets, but strain 3 seldom infected the resistant beet used. On the other hand, strain 1 infected a high percentage of resistant beets and induced obvious symptoms. Strains 2 and 4 induced mild symptoms in susceptible beets, but strain 4 infected only a small percentage of the resistant beets. On the contrary, strain 2 infected a high percentage of the resistant beets, but induced mild symptoms. The highly-resistant beet variety, known as 1167, showed distinctive reactions to each of the 4 strains of virus and the differences were highly significant statistically. Tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.) were not infected by strains 2 or 4. Plantain (*Plantago erecta* Morris), peppergrass (*Lepidium nitidum* Nutt.) and Great Northern variety of bean (*Phaseolus vulgaris* L.) were not infected by strain 2. Susceptible beets infected with the less virulent strains were not immunized against the more virulent strains, but appeared rather to be rendered more susceptible to severe injury by such prior infection.

M33. Control of Plant Virus Diseases in California. HENRY H. P. SEVERIN, University of California, Berkeley.

Several methods are available for controlling curly top of the sugar beet and the beet leafhopper, *Eutettix tenellus* (Baker). One depends on the use of a definite planting schedule for sugar beets. This schedule, designed to avoid curly top in natural breeding areas, is related to the spring and autumn dispersal of the insects. Another method employs a variety of sugar beet, developed by the United States Department of Agriculture, which is resistant to curly top. Other lines of attack designed to control the beet leafhopper are (1) control by spraying (based upon the fact that the leafhoppers are forced to congregate in large numbers upon vegetation in dry washes during the autumn), (2) control by destroying the principal summer host plant, the Russian thistle (*Salsola kali-tenuifolia*), by summer hoeing, dragging, or disking, and (3) control through the ultimate elimination of the Russian thistle by preventing its spread and reducing its seed supply by burning the mature thistle stands late in the autumn before the plants are torn loose and start rolling.

A practical method for combating western celery mosaic involves adopting a celery-free period in the fields varying from 3½ to 5 months

and in the greenhouses from 1 to 1¾ months. All fields in the restricted area are plowed and all plants not previously harvested are destroyed. No celery is allowed to be shipped into these districts from other localities.

In Colorado it has been demonstrated that peach mosaic may be controlled by the removal of diseased trees. Similar methods are now being used in California.

M34. Detection of the Virus of Poliomyelitis in the Nose and Throat and Gastrointestinal Tract of Humans and Monkeys. SIDNEY DAVID KRAMER, Long Island College of Medicine, Brooklyn, N. Y.

Nasal washings and stools from 20 patients convalescent from poliomyelitis, and nasal washings, scrapings and intestinal contents from 7 monkeys sacrificed at the height of the experimental disease, were studied. These materials were treated with ether and concentrated *in vacuo*. The concentrated materials were inoculated intracerebrally and intraperitoneally into normal monkeys. Animals presenting suggestive symptoms were sacrificed and serial passages to second animals were made. Positive detections of the virus were conceded when the monkeys presented symptoms and histologic findings compatible with experimental poliomyelitis.

The virus was detected in 4 instances from human sources and in 1 instance from a monkey. Of the 4 human strains, the first was obtained from the feces of a child 7 days after onset of illness. The second strain was obtained from the nasal washings of a child 5 days after onset. The third and fourth human strains came from the same patient 9 days after onset, from the nasopharynx and the feces, respectively. The single strain recovered from a monkey was obtained from the gastrointestinal tract.

In view of the normal swallowing of oral and nasal secretions, it is suggested that recovery of the virus from the human gastrointestinal tract need not alter the concept that the virus normally enters the body by way of the upper respiratory tract.

M35. Influence of Sterile Inflammation on the Nasal Portal of Entry in Poliomyelitis. P. F. CLARK, K. E. LEMMER AND A. F. RASMUSSEN, University of Wisconsin Medical School, Madison.

It is now commonly admitted, on the basis of much evidence, that the usual portal of entry in poliomyelitis is through the olfactory mucosa.

Lennette and Hudson found that sectioning of the olfactory tracts of monkeys prevented the disease, even when the intravenous route, with large doses of virus, was employed. Similarly, Armstrong protected animals against intravenous injections by treating the nasal mucosa with picric acid.

In a series of 8 *Macacus rhesus* monkeys, the olfactory tracts of 4 were sectioned, while 4 were left normal. After several weeks had elapsed for recovery, a sterile inflammation was produced in 2 of the sectioned animals and in 2 of the normal controls, by injecting 1 cc. of 1 per cent starch paste into each frontal lobe, and 2 cc. of normal horse serum intraspinally by lumbar puncture. On each of the next 3 days, each of the 8 animals were injected intravenously with 10 cc. of supernate from a 5 per cent centrifuged suspension of glycerinated cord (30 cc. total dose). Only the 4 monkeys with the sterile inflammation developed poliomyelitis.

It seems probable that this inflammation caused sufficient injury to the blood central-nervous-system barrier to permit the virus to pass directly to the brain and cord. It is clear, therefore, that an otherwise subinfective intravenous dose was rendered infective by a sterile inflammation, whether the olfactory tracts were sectioned or not. The intact olfactory tract was not, apparently, a determining factor.

M36. Relation of the Concentration of Virus to the Pathogenesis of Poliomyelitis. E. W. SCHULTZ AND L. P. GEBHARDT, Stanford University, Calif.

Recent investigations on the kinetics of bacteriophagy indicate that bacteriophage may be formed by intact living bacteria and that lysis may not occur until a certain "critical" concentration of the lytic principle has been reached. We have made observations which suggest that in poliomyelitis there is a similar relationship. Titration studies during the preparalytic stage of the experimental disease show that the virus may reach high concentrations in certain portions of the nervous system without producing conspicuous nerve-cell damage and may attain a high concentration in the cord before paralysis is manifested. In fact, its concentration in the cord at the time of paralysis may be lower than that reached in the olfactory bulbs and hypothalamus, in which less nerve-cell damage seems to occur.

Briefly, our observations suggest that the concentration of virus is not necessarily directly related to nerve-cell destruction and that virus can be formed in large amounts in certain areas without producing

extensive nerve lesions; also that paralysis is due not so much to a particular mobilization of the virus in the cord and medulla as to an inherently greater susceptibility of anterior horn-cells.

It seems probable that, like strains of bacteriophage which despite serial passage remain of low titer and low lytic power, there are "low titer" strains of poliomyelitis virus, which although not lacking in invasiveness may be unable to build up a concentration of virus high enough to damage nerve-cells. Naturally acquired immunity may be based largely on exposure to such strains.

M37. Variations in Strains of Poliomyelitis Virus. FRED D. STIMPERT AND JOHN F. KESSEL, University of Southern California School of Medicine and Los Angeles County Hospital, Los Angeles.

Five strains of poliomyelitis virus isolated in Los Angeles have been compared with two Eastern strains, the M.V. strain and one isolated recently by Dr. L. W. Aycock in Boston. The comparison was made by means of cross-neutralization tests using sera of monkeys recovered from infection. A minimum of 10 animals was used in an experiment with each virus. The final suspension of virus in each injected dose of serum-virus mixture, as determined by the titration of pools, contained 10 minimal lethal doses. The dilution of serum used was 1–10.

Wide ranges of virulence were noted in animals infected with different strains, such differences being independent of the immunologic differences shown by neutralization tests. It is indicated that the development of neutralizing antibodies against homologous virus in the serum of monkeys recovering from infection was quite regular, and that the sera of animals recovering from the less severe disease demonstrated, in general, greater neutralizing power.

A closer relationship is noted between strains from the Los Angeles area than between these and the strains from other areas. Immunologic differences, however, do occur within the group of recently isolated strains from Los Angeles. Differences are also apparent between recently isolated strains and the M.V. strain, and between the M.V. strain and the strain from Boston. Neutralization may occur in one direction without reciprocation.

In summary, it would appear that these indications of immunologic variations are due to differences in antigenic structure rather than to the fact that a certain strain is a recently isolated one, an old passage strain, or one related to a particular epidemic.

M38. Neutralization Test with the Sera of Poliomyelitis Patients from the Recent Epidemic in Los Angeles. JOHN F. KESSEL, FRED D. STIMPERT AND ROY T. FISK, University of Southern California School of Medicine and Los Angeles County Hospital, Los Angeles.

Sera collected from poliomyelitis patients in the Los Angeles County Hospital during the period from 1934 to 1936 were employed in neutralization tests with the M.V. strain of virus from the Rockefeller Institute, the McK strain isolated in Los Angeles in 1935, the St. strain isolated in Los Angeles in 1936 and the Bo strain isolated by Aycock in Boston in 1936.

Of 41 onset and convalescent sera compared, 28 per cent of the onset sera and 50 per cent of the convalescent sera neutralized M.V. virus, while 48 per cent of the onset sera and 84 per cent of the convalescent sera neutralized the local McK virus.

In comparing the convalescent sera from 37 children and from 35 adults, it was found that 38 per cent of the children and 70 per cent of the adults neutralized the M.V.virus, while 70 per cent of the children and 97 per cent of the adults neutralized the local McK virus.

In comparing the convalescent sera of 38 patients with mild symptoms and 29 with severe symptoms, it was found that 53 per cent with mild symptoms and 55 per cent with severe symptoms neutralized M.V. virus, while 72 per cent with mild symptoms and 92 per cent with severe symptoms neutralized the McK virus.

In comparing the convalescent sera with the 4 strains of virus, it was found that 40 per cent neutralized the M.V. strain, 50 per cent neutralized the St. strain, 30 per cent neutralized the Bo strain, and 90 per cent neutralized the McK strain. Variations of neutralization results, using the same sera but different strains of virus, are thus noted.

M39. Immunological Reactions in Poliomyelitis. SIDNEY RAFFEL, Stanford University, Calif.

Studies were made in an attempt to demonstrate reactivity between poliomyelitis immune sera and the virus or its products in nervous tissue. These investigations were directed toward a further analysis of the immunologic properties of the virus, as well as toward simplification of the identification of infection or the recognition of acquired immunity. The procedures involved the testing of virus and normal cord suspensions, extracts and fractions, variously concentrated, by active and passive

sensitivity tests in guinea pigs and monkeys; also by precipitation and complement-fixation tests and by Shwartzman reactions in rabbits.

With most of the methods employed, there was no indication of an immunologic distinction between infected and normal cord preparations. Specific reactivity has been noted, however, in a limited number of sensitivity tests carried out with ultracentrifuged cord sediments. Since such sediments have previously been shown to contain relatively high concentrations of the virus, further confirmatory work is being carried on. It is planned, also, to determine whether the virus itself is responsible, or whether some other cord element takes part in the reaction.

M40. Neutralization and Complement-Fixation Tests with Four Neurotropic Viruses and Human Sera Collected in California during 1937 and 1938. BEATRICE HOWITT, Hooper Foundation for Medical Research, University of California, San Francisco.

Sera were collected in California from regions where cases were reported as having recovered from either a typical encephalitis or a "polio-encephalitis" during the summer of 1937. A few had been diagnosed during 1936.

The neutralization test showed that 40.3 per cent of 57 sera were positive against St. Louis encephalitis virus. All but one of the positive cases resided in some portion of the two large central California valleys, the majority around Fresno and Tulare (30 cases with 56.6 per cent positive). The same sera were also tested against the viruses of poliomyelitis, of lymphocytic choriomeningitis and of Japanese B encephalitis. Tests with the two latter were negative, but 16 (51.6 per cent) of 31 sera neutralized the virus of poliomyelitis.

About 76 of the sera from a group of cases diagnosed as having had poliomyelitis without residual paralysis in Tulare County since 1934 gave positive neutralization against poliomyelitis virus. A certain number of them were also positive against St. Louis encephalitis virus, thus indicating the presence of this disease for a longer period than had been suspected. No positive tests for this latter virus were obtained with sera from individuals who had had typical poliomyelitis with residual paralysis. Normal sera, with the exception of that of a nurse from Fresno, were negative.

It was found feasible to use the complement-fixation test as a supplementary aid in the diagnosis of St. Louis encephalitis. Many of the sera showed definite correlation between positive neutralization and

positive complement fixation. All normal sera and those from paralytic cases of poliomyelitis, however, have been negative.

M41. Production of Antiserum for Equine Encephalomyelitis. EDWARD RECORDS AND L. R. VAWTER, University of Nevada, Reno.

Antisera of high virus-neutralizing titer will probably be needed for some time to come as an aid in the treatment of clinical cases of equine encephalomyelitis. Even after a prolonged course of immunization by the subcutaneous injection of virus into horses, the response is poor or variable and the sera of only a few animals attain a titer considered satisfactory for clinical use. We have recently observed that a single intravenous injection of a large dose of equine encephalomyelitis virus into immune horses results in a ten-fold rise of antiviral titer in 10 days. So far, we have encountered no fatalities or unduly severe reactions in the injected horses following this procedure. Titration of the virus-neutralizing power of individual and pooled sera from hyperimmune horses has been conducted on both 10-day chick embryos and guinea pigs. The chick embryo is considered equally, if not more, dependable than guinea pigs. The use of the embryos, moreover, is decidedly more economical, and the procedure is more rapid.

M42. Heterogeneity of Coliform Bacteria as Shown by the Absorption of Bacteriophage. PHILIP L. CARPENTER, Iowa State College, Ames.

On the basis of recent work, it may be assumed that the specificity of the absorption of bacteriophage is dependent upon the factors that determine serological specificity. Therefore, an attempt was made to separate 127 strains of coliform organisms into groups based on the absorption of anti-coli and anti- "Citrobacter" bacteriophages. The coliform strains were representative of *Escherichia*, *Aerobacter*, "Citrobacter" and Voges-Proskauer-positive "intermediates". The 4 groups separated by absorption did not correspond even closely to the grouping of the strains on a physiological basis. There were indications of marked serological heterogeneity.

M43. Specificity of Bacteriophage for Strains of the Escherichia-Aerobacter Group and for Certain Other Members of the Bacteriaceae. M. J. POWERS, MAX LEVINE AND C. S. MCCLESKEY, Iowa State College, Ames.

Bacteriophages were isolated from sewage and packing-house waste

against 11 strains of *Escherichia;* 8, *Aerobacter;* 8, *Escherichia-Aerobacter* intermediates; 2, *Proteus;* 1, *Salmonella;* 2, *Eberthella;* 1, *Alkaligenes;* 1, *Klebsiella;* 2, *Flavobacterium;* 2, *Serratia;* and 1, *Achromobacter.* The bacteriophages were purified by repeated "picking" from isolated plaques on agar in Petri dishes. Tests for lysis were made by smearing a young bacterial culture over the surface of agar in a Petri dish, and then streaking the bacteriophage to be tested across the surface of this inoculated substrate. Each bacteriophage was tested against the strains listed above, also against a considerable number of additional cultures of the *Escherichia-Aerobacter* group and other bacteria.

Bacteriophages isolated against strains of the genus *Escherichia* showed a marked tendency to lyse organisms in that genus, but not strains of the genus *Aerobacter,* and *vice versa.* Bacteriophages for *Escherichia-Aerobacter* intermediates showed a moderate tendency to specificity for that group of cultures. Rather specific bacteriophages appear to exist for bacteria in each of the genera *Proteus, Flavobacterium, Serratia* and *Klebsiella.* Some bacteriophages attacked only *Escherichia-Aerobacter* strains, while others attacked both these strains and certain intestinal pathogens as well.

M44. Observations on a Mixed Strain of Staphylococcal Bacteriophage.
EUGENE MAIER, Florida Medical Center, Venice.

A mixed *Staphylococcus aureus* bacteriophage has been employed for about a year for the production of "specific" bacteriophage for use in the treatment of cases of osteomyelitis. In a recent case originating in the calcaneus, all attempts to obtain a "specific" bacteriophage were made in vain. The bacteriophage failed to reproduce, so that none was demonstrable after a few passages. At this point, the widely known and used *Staphylococcus aureus* (205), Department of Agriculture, Washington, was found to be readily lysable. This staphylococcal strain has been kept on agar for a period of 10 years. An attempt was made to obtain lysis from the osteomyelitic strain by pooling stock bacteriophage and the bacteriophage recovered from *Staphylococcus aureus* (205). Complete lysis occurred in the second transfer, the tubes of broth remaining clear for 12 hours. The addition to the stock bacteriophage of dimethyl-benzyl-ammonium chloride in a concentration of 1:100,000 apparently had no deleterious effect on the lytic principle.

M45. Further Studies on the Development of Immunity by Various Tissues.
LORE MARX AND REUBEN L. KAHN, University of Michigan Hospital, Ann Arbor.

Rabbits were immunized by an intravenous injection of horse serum and, after different intervals, various tissues of the rabbits were tested for their capacity to retain injected antigen. These tests were made by the simultaneous injection of horse antitoxin into the tissue to be tested and of a standard dose of homologous toxin subcutaneously. The excess of antitoxin necessary to protect a horse serum-immune animal as compared with the protective dose for a normal animal was the measure of the amount of horse serum protein retained in the tissue. Highest retaining capacity was found in one or another tissue, depending upon the immunizing dose and the interval following immunization.

After immunization with 0.001 cc. of horse serum, the skin and peritoneum developed a high capacity to retain horse antitoxin. The response of the peritoneum was more rapid and marked, but subsided sooner than the cutaneous response. Skeletal muscle retained but very little antitoxin. If 1 cc. of horse serum were used for the immunizing injection, the retention of antigen in all three tissues was higher and lasted longer, but appeared more slowly, and the skin showed a higher retaining capacity than the peritoneum.

M46. A Study of Natural and Acquired Immunity to Staphylococcal Toxin in Monkeys. CHARLES WEISS AND J. D. TARANIK, Mount Zion Hospital, San Francisco, Calif.

The staphylococcal anti-α-hemolysin of the serum of 56 normal monkeys (*Macacus irus* and *Macacus mulatta*) varied from 0 to 25 international units. Intraperitoneal injection of from 10,000 to 20,000 units of Squibb's staphylococcal antitoxin (concentrated horse serum) raised these titers within 48 hours after administration to from 80 to 150 units, the average being about 100. Antitoxin was eliminated rapidly from the blood stream of the monkeys. Thus, there were precipitous drops in titers from 24 to 48 hours after they reached a peak. After a month, the titers had returned approximately to their original levels.

Intradermal or intrabronchial immunization with toxin protected monkeys against several minimal lethal doses of this antigen, when administered intrabronchially, and caused a marked reduction in the severity of cutaneous reactions to test doses. There was much variation in the capacity of animals to develop antihemolysin. The titers reached varied directly with the original (natural) levels.

As in the Schick reaction with diphtheria toxin, there was no fixed antitoxin titer (anti-α-hemolysin) above which animals showed an absence of dermonecrosis, and below which they were positive in this respect. Nevertheless, there was a much higher level of circulating

antihemolysin (10 to 60 units) in groups of negative than in groups of positive monkeys, both in their normal state and after active or passive immunization.

M47. Studies on Anaphylaxis in the White Mouse. RUSSELL S. WEISER, ORVILLE GOLUB AND DOROTHY HAMRE, University of Washington, Seattle.

The intraperitoneal injection of multiple, spaced, sensitizing doses of diluted egg-white or rabbit serum serves to sensitize white mice so that anaphylaxis is fatal when the shocking dose is administered either intravenously or intraperitoneally. Fatal anaphylaxis was produced as early as the 12th day after the beginning of the sensitization treatment and the 4th day following sensitization. It persisted rather uniformly until the 15th or 20th day, when a drop in sensitivity occurred. By the 30th day fatal shock was produced in only an occasional animal, and by the 45th day anaphylaxis was absent. A single large dose of egg-white was not efficient for anaphylactic sensitization. Anaphylaxis was not as constant among very young mice as among older animals. Repeated sub-lethal doses of antigen served to desensitize actively sensitized mice. Fatal anaphylaxis was not produced in mice previously treated with the sera of sensitized rabbits or mice. In a second phase of the work, adrenalectomized mice were used. Such mice were extremely susceptible to shock, yet they could be desensitized. Although there was an apparent correlation between anaphylaxis and serum precipitins during the early period following sensitization, fatal anaphylaxis was often produced months later, when precipitins were not demonstrable. Passive sensitivity produced with rabbit serum was of short duration and usually disappeared by the 30th day.

M48. Incidence of H and O Agglutinins for Typhoid and Paratyphoid Bacilli in the Sera of Persons with no History of Enteric Disease. JOHN PHILLIPS AND H. J. SEARS, University of Oregon Medical School, Portland.

More than 200 specimens of serum from individuals with no history of enteric disease have been examined for the O and H agglutinins of the typhoid bacillus and of two of the paratyphoid bacilli. Seventy per cent of the sera showed no agglutination with any antigen in a dilution of 1:20, while 30 per cent were positive for one or more of the antigens at this or higher dilutions. The incidence of agglutination

was higher for the O antigens than for the H, and agglutinins were more frequently demonstrated for the paratyphoid B organism than for typhoid or paratyphoid A bacilli. A small group of sera from vaccinated persons showed variable results.

M49. Some Serological Relationships of the S, R and G Phases of Bacillus salmonicida. D. C. B. DUFF, University of British Columbia, Vancouver, Canada.

Cultural phases S, R and G of *Bacillus salmonicida* have been established previously by the author (1937). Subsequent work has attempted to elicit the serological relationships of these phases, both within homologous cultures and among heterologous strains. Methods so far have been confined to reciprocal agglutinin absorptions and to precipitation reactions. Antigen has always been killed with 0.2 per cent formaldehyde, and suspensions for agglutination titration have been made in 0.05 per cent sodium chloride. Invariably R antigen showed a much lower absorptive capacity, both for R and for S agglutinin, than did S strains.

In each of 14 *Bacillus salmonicida* strains, reciprocal absorptions showed antigenic composition of the form $R = S + n$. In 5 other strains, $S_1 = R_1 + n_1$. With all 19 strains, the G phase possessed some antigens common to both R and S, together with some individual antigen. Comparison of R phases of different strains showed slight antigenic differences, in some cases. A more marked variation occurred among the S phases of the same strains. Antigenic variation in S strains, as determined by the experimental methods noted, could not be correlated with virulence. Sixteen morphologically true single-cell S strains were pathogenic to goldfish, but possessed varying virulence, 3 strains being non-pathogenic. No R or G phase of any strain ever produced disease. Precipitation reactions in general support the agglutinin-absorption findings.

M50. Further Studies on the Purification and Analysis of Diphtheria Toxin. AUGUSTUS B. WADSWORTH AND MARY W. WHEELER, New York State Department of Health, Albany.

Previous papers have described the production of toxin in a purely synthetic medium and its purification by ultrafiltration. The present report will record the results of further studies on the preparation of toxic and non-toxic fractions from synthetic medium, and on the puri-

fication of toxin by ultrafiltration. Investigations of the biological, chemical, and physical properties of these fractions will be described.

M51. A Study of the Quantitative Complement-Fixation Test in Tuberculosis. AUGUSTUS B. WADSWORTH, ELIZABETH MALTANER AND FRANK MALTANER, New York State Department of Health, Albany.

The complement-fixation test with tubercle antigen has been completely revised to provide a quantitative titration of the activity of the serum. The new procedure is based on linear relationships which have been demonstrated in previous studies between complement and serum or antigen, when all three reagents are used in amounts to give maximal interaction. The present paper will record the results obtained in quantitative tests of specimens from active and inactive cases of tuberculosis in various stages of advancement.

M52. Complement-Fixation Reactions with Pertussis Undenatured Bacterial Antigen. H. M. POWELL AND W. A. JAMIESON, Lilly Research Laboratories, Indianapolis, Ind.

In view of the importance of the native-protein content of Pertussis Undenatured Bacterial Antigen, work was undertaken to correlate biological methods of testing with chemical standardization. Positive complement-fixation results were observed to be specific with the antigen, and appear to be useful in assaying the native-protein content as determined by chemical methods. Such biological tests necessitate but a single day for testing as compared to a period of 6 or 8 weeks for conducting mouse tests.

M53. Treatment of Antiserum to Remove Serum-Sickness-Causing Factor. LLOYD R. JONES AND MOYER S. FLEISHER. St. Louis University School of Medicine, St. Louis, Mo.

Phenolized and non-phenolized portions of a lot of tetanus antitoxin (whole horse serum) were subjected to various modifications of a method for the removal of the factor causing serum sickness in the rabbit. The method involved the addition of dilute alkali to the serum, application of heat at 50° or 55°C. to the mixture for one or one and one-half hours, and subsequent neutralization with an equivalent amount of acid. After restoring the diluted serum to its original volume by evaporation, it was tested in rabbits for serum-sickness-causing activity and titrated

for antitoxic content by protection tests in guinea pigs against known doses of toxin.

The effect of varying the concentration of alkali, the temperature, and the period of application on the removal of serum-sickness-causing factor and on the destruction of antitoxic activity will be described. In dealing with the sample of non-phenolized serum, it was found possible to adjust the method to the particular requirements of the lot of serum under examination in such a way as to remove all of the serum-sickness-causing activity and yet to retain 80 per cent of the original antitoxic content. Phenolized serum, however, required somewhat more drastic treatment (*e.g.*, higher concentrations of alkali) for the removal of serum-sickness-causing activity. Consequently, there was a somewhat greater destruction of antitoxin.

M54. The Protective Action of Egg-Albumen when Administered Orally with Clostridium botulinum (Type C) Culture to Pigeons. MILLARD F. GUNDERSON, University of Nebraska College of Medicine, Omaha.

The toxic potency of the culture of *Clostridium botulinum* (Type C) employed in these studies was such that 0.001 cc. injected into each of 20 pigeons caused their death within 24 hours. When fed to 10 birds in 1 cc. doses, this culture killed 3 within 9 hours, 3 within 18 hours, and 1 within 36 hours. One pigeon was sacrificed while lethargic after 144 hours and 2 displayed no symptoms. When 3 cc. amounts of this culture were fed to each of 10 birds, all 10 died within 24 hours.

Birds were fed 3 cc. doses of culture mixed with 0.5 cc. amounts of proteins of various kinds. Antitoxins and normal sera had a detoxifying effect. In 3 instances the birds survived. In the case of a single bird death was delayed for 12 days. Egg-albumen in amounts of 0.5 cc. was mixed with each 3 cc. dose of culture and this mixture was fed to each of 10 birds. One survived, and the rest lived for periods varying from 8 to 15 days.

The ingested toxin-protein complex could be broken up by drenching the birds with 2 cc. amounts of a 5 per cent sodium carbonate-saturated sodium sulfate solution. A series of 10 poisoned birds so treated showed a shortening of the period between dosage and death. Four died within 24 hours, 2 within 12 hours, and 2 lived for 7 days.

Gelatin and heated serum gave only indifferent results. In similar experiments with Type A toxin little evidence of binding was secured.

The relation of these observations to naturally occurring avian botulism is considered.

M55. The Susceptibility of the Antigenic Types of Staphylococci to Several Protective Enzymes. RICHARD THOMPSON AND EDUARDO GALLARDO, JR., College of Physicians and Surgeons, Columbia University, New York City.

In a previous communication we reported that pathogenic staphylococci of Type A are able to grow in human whole blood and serum to a much greater extent than are the other types. In view of the suggested relationship between pathogenicity and resistance to protective enzymes, the susceptibilities of the different types of staphylococci to several agents have been compared. Purified egg-white lysozyme, human tears, rabbit serum and rabbit-leucocyte extracts have been used. The tests were done by comparing the concentrations of the various agents required to produce visible lysis of suspensions of the different organisms and to inhibit their growth in broth or on agar. *Micrococcus lysodeikticus* was very susceptible to all of the agents. Of the staphylococci, Types B and C showed definite degrees of visible lysis by egg-white lysozyme, tears and leucocytic extracts. Serum inhibited the growth of all but the Type A staphylococci which were affected only by leucocytic extracts. However, they were markedly inhibited by these extracts. It is uncertain to what extent the differences in the agents are qualitative rather than quantitative; but it is indicated that the leucocytic agent, at least, is qualitatively different from egg-white lysozyme.

M56. Proteinases and Peptidases during Various Phases of Experimental Tuberculosis in Rabbits. CHARLES WEISS AND A. KAPLAN, Mount Zion Hospital, San Francisco, Calif.

Employing hemoglobin as a substrate, it was shown that polymorphonuclear neutrophilic leucocytes, monocytes and epithelioid cells contain a cathepsin with optimal activity at pH 3, (range pH 1 to 5.5); also a dipeptidase hydrolyzing alanyl-glycine at pH 8. Carboxypeptidase splitting chloracetyl-tyrosine at pH 8 is lacking. The monocytes may, however, be differentiated from myelocytic leucocytes, since only the latter can split alanyl-glycine at pH 5.5 and hydrolyse gelatin and casein at pH 8. Since epithelioid cells are derived embryologically from monocytes, it is of interest to note that they have an enzyme-pattern similar to the latter, but not to polymorphonuclear neutrophilic leucocytes.

Tissues of rabbits (normal, allergic and immune) were examined for their proteinase and peptidase content. The data will be presented and discussed.

AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

A1. Distribution and Activity of Azotobacter Organisms in Arizona Soils. W. P. MARTIN AND R. A. AYERS, College of Agriculture and Agricultural Experiment Station, University of Arizona, Tucson.

Some 76 cultivated soils of Arizona have been examined for the presence of *Azotobacter* organisms. Only 9 samples did not contain the bacteria; these had a total salt content of over 3,000 p.p.m. The bacteria were very active in these soils, as indicated by an average fixation of over 22 mg. of nitrogen per gm. of soil tested. Curie's method was used in these tests and the incubation period was only 4 days. The amount of nitrogen fixed per gm. by *Azotobacter* organisms from some Iowa soils, in which a highly significant increase in the growth of timothy resulted from an inoculation with a vigorous growth of the organisms after the soils had been improved with lime, was but 12 mg. of nitrogen per gm. of soil tested. Consequently, in the cultivated soils of Arizona the amount of nitrogen fixed by *Azotobacter* organisms may aid materially in the maintenance of the soil's nitrogen supply and may help to account for the large amount of nitrate-nitrogen found in the drainage waters; this has exceeded 300 p.p.m. in some cases.

The range soils of Arizona were generally lacking in *Azotobacter* organisms, the bacteria having been found in only 6 of 23 samples tested. The amount of nitrogen fixed per gm. in these samples averaged about 3 mg. per gm. of soil tested, which indicates that the bacteria in these samples were not very active as compared with those in the cultivated soils.

A2. Some New Non-Symbiotic Nitrogen-Fixing Bacteria from the White Sands of New Mexico. CHARLES B. LIPMAN, University of California, Berkeley.

In a survey of the microbial flora of the White Sands of New Mexico, which are an almost pure gypsum formation with shifting dunes, it was discovered that the sands inoculated into appropriate media yielded marked fixation of nitrogen. In hundreds of examinations it has been impossible to find any definite *Azotobacter* organisms in the flora re-

sponsible for the fixation. Therefore, a special study covering a period of two years has been made of the nitrogen-fixing bacteria occurring in the White Sands. As a result of these studies several forms of bacteria have been discovered which seem to be different from any non-symbiotic nitrogen-fixing bacteria thus far described in the literature. A brief account of these new forms and of their nitrogen-fixing powers will be given in the paper.

A3. The Activity of Azotobacter Organisms under Field Conditions. S. C. VANDECAVEYE, State College of Washington, Pullman.

Using the silica-plate method, periodical counts of *Azotobacter* organisms were made for several years on representative soil samples obtained fresh from the field. A marked seasonal fluctuation in the numbers of *Azotobacter* organisms and a gradual disappearance of these bacteria from certain soils which once supported an active flora were observed.

Berkefeld-N-candle filtrates were obtained from soil added to an *Azotobacter*-nutrient solution and incubated at 28°C. for 36 hours. These had an inhibiting effect on the growth of *Azotobacter* organisms on agar plates when proper amounts of the filtrates were applied to 24-hour liquid cultures of *Azotobacter* strains. The inhibiting effect was expressed in the form of plaque-like formations. A few attempts to demonstrate transmissible lysis by serial transfers of a limited number of strains of *Azotobacter* were unsuccessful, but inoculation of the affected soil with different species and strains of this organism disclosed a marked difference in the tolerance of various strains to the inhibitng factor in the soil.

A4. Is the Ability to Metabolize Atmospheric Nitrogen a Constant Characteristic of Azotobacter Organisms? P. L. GAINEY, Kansas State College, Manhattan.

A limited percentage of strains of *Azotobacter chroococcum*, when grown for long periods on an agar medium high in nitrate-nitrogen, will lose the ability to grow on a "nitrogen-free" agar. Certain of these strains, when supplied with limited quantities of fixed nitrogen, rapidly regain the ability to grow upon "nitrogen-free" agar. Other strains seem to lose permanently the ability to grow upon a "nitrogen-free" agar, all subsequent growth being directly proportional to the quantities of fixed nitrogen supplied in the medium. Failure to grow upon a "nitrogen-free" agar is due apparently to an inability to metabolize free atmospheric nitrogen.

A5. Strain Variation and Host Specificity of Rhizobium within the Cowpea Cross-Inoculation Group. O. N. ALLEN AND ETHEL K. ALLEN, University of Hawaii, Honolulu.

A study has been made of the infective and effective abilities of 54 strains of *Rhizobium* isolated from 28 species of leguminous plants when used as inocula for 20 plant members of the cowpea cross-inoculation group. Results have been based upon three replicated greenhouse experiments which were conducted under approximately the same meteorological conditions.

Marked differences were noted in the responses of the plants to the various inocula. Only 25 per cent of the strains produced nodules upon all of the test plants. In regard to benefits derived from the inoculation, 48, or 89 per cent, of the strains were beneficial upon more than 10 of the species of plants. Four strains benefited the growth of 19 species. Ten of the test plants were nodulated by all of the inocula. *Vigna sinensis* was the most susceptible, inasmuch as each strain of *Rhizobium* not only produced nodules, but enhanced its growth; while the other extreme was represented by *Phaseolus lunatus* upon which only 22 strains produced nodules, none of which were beneficial.

The results suggest that variations in the infectiveness and effectiveness of *Rhizobium* strains from plants of the cowpea group may be of a greater magnitude than those previously recorded for the other cross-inoculation groups.

A6. A Cytological and Histological Study of the Root-Nodules of the Peanut (Arachis hypogaea L.). O. N. ALLEN AND ETHEL K. ALLEN, University of Hawaii, Honolulu.

Inoculation of peanut plants with good and poor strains of nodule-forming bacteria has shown two types of nodules: one, comparatively large, beneficial nodules located on the upper central tap-root system; the other, tiny, innumerable, non-beneficial nodular swellings, scattered throughout the root-mass, and most readily seen when immersed in water. Both types were located in the axils of the roots.

Results obtained do not indicate any differences in the origin of these nodular types, inasmuch as each arises apparently from infection within the ruptured axillary tissue at the point of emergence of the lateral roots. Paraffin sections of the infected areas made prior to a macroscopic enlargement of the nodular tissue show a distinct proliferation of cells originating opposite protoxylem strains in the axils of the lateral roots. Such proliferations are at first completely enclosed within the pericycle of the roots. In the early stages of formation the nodular swelling is

entirely meristematic; later a ring of endodermis is formed, and as growth progresses a periderm layer encloses the rapidly growing nodular mass. The parenchymatous cells of the root-cortex appear torn and partially absorbed due to the outward growth of the nodular tissue, but are retained in later stages as a sheath distinct from the nodular cortex. It appears that these nodules arise endogenously from the same layers of cells as do lateral roots. The bacterial phase of the *Rhizobium* organisms within the nodules presents morphological variations different from those heretofore described in nodules of other species.

A7. Combination of Mixed Groups of Rhizobium in a Single Culture. V. S. Bond, Kalo Inoculant Co., Quincy, Ill.

Cultures of root-nodule bacteria are commonly prepared with organisms of but one plant group, *i.e.*, the culture is applicable only to those leguminous seeds with which a single type of *Rhizobium* will cross-inoculate. In practice, as many legume inoculants must be prepared and distributed as there are common cross-inoculation groups. The desirability of combining the organisms representative of two or more groups in a single unit led to the present investigation.

Combination cultures were checked according to standard experimental technic by growing the inoculated plants in sterile sand supplemented with all of the essential nutrients except nitrogen to determine whether they satisfied the requirements of a satisfactory single-group culture. These requirements demand that, at any time within the expiration date, sufficient organisms of the proper type be present to produce an adequate nodulation and a high nitrogen-fixing efficiency to yield maximal benefit to the host plants. Nodule formation was checked after growth for 3 or 4 weeks and the ability of the organisms to fix atmospheric nitrogen was measured by analyzing full-grown plants for total-nitrogen content. In every experiment, uninoculated controls to detect possible contamination and controls inoculated with standard single-type culture to afford direct comparison were included.

The data indicate that practical legume inoculants containing two or more culture groups combined in a single unit may be prepared which compare favorably with standard single-group cultures, with respect to both nodule formation and efficiency of nitrogen fixation.

A8. Growth-Factor Requirements of Rhizobium trifolii. P. M. West and P. W. Wilson, University of Wisconsin, Madison.

Studies on the growth-factor requirements of *Rhizobium* strains indi-

cate that the heat-stable substance extracted from *Azotobacter* cultures and described by Allison and Hoover provides the nodule organism with a constituent greatly increasing the initial rate of growth. Although this material is capable of inducing growth in a medium of unfavorable oxidation-reduction potential, it appears that its stimulative value is greater than that which could be obtained by the simple addition of reducing substances. While effective in accelerating the rate of cell multiplication, it is not indispensable to growth as was previously believed.

Although *Rhizobium* strains do not appear to synthesize this heat-stable stimulative material found in *Azotobacter* or yeast extracts, further studies have revealed that a more active, heat-labile substance is produced by the organisms themselves when growing in a purely synthetic medium. Even normal loop transfers carry sufficient of this factor from the previous culture to allow a satisfactory growth to occur in a medium of the proper oxidation-reduction potential. Through special precautions it has been possible to prepare inocula relatively free from the old-culture material. This has resulted in a marked decrease or in a complete failure on the part of the cells to multiply. By suspending such an inoculum in an unheated cell-free filtrate from a growing culture, however, growth takes place normally. The stimulative effect of small amounts of unheated culture-filtrate on the growth of the organism has also been observed by a giant-colony technic modified for quantitative studies.

A9. The Presence and Significance of Oxalacetic Acid in Plant Tissues. ORVILLE WYSS AND P. W. WILSON, University of Wisconsin, Madison.

Recently, it has been suggested that oxalacetic acid may play an important intermediary rôle in biological nitrogen fixation. The occurrence of the acid in leguminous plants actively fixing nitrogen has been used in support of this hypothesis. The established importance of oxalacetic acid in the four-carbon dicarboxylic acid cycle of animal-tissue respiration suggests the importance of a study of these acids in plant tissue.

Oxalacetic acid was determined in grasses and cereals and other types of non-leguminous plants. Legumes which were fixing nitrogen and others supplied with combined nitrogen were compared as to content of the keto-acid. Determinations were conducted by both a colorimetric and a manometric method. The results are presented and interpreted

in the light of their significance for the biochemistry of biological nitrogen fixation.

A10. The Influence of Cropping on the Nitrogen-Fixing Capacities of the Soil. J. E. Greaves and A. F. Bracken, Utah Agricultural Experiment Station, Logan.

When soils from wheat land and adjacent virgin land from Juab and Cache Counties, Utah, were inoculated into a synthetic medium, the gains in nitrogen from the cropped soils were greater than from the virgin soils. The nitrogen gains made by the Cache soils were considerably greater than those of the Juab soil. This was due to two factors: (1) the Cache soils contain a larger quantity of carbonaceous material than the Juab soils; (2) practically all of the Cache soils carry a rich *Azotobacter* flora, whereas these organisms are generally absent in the Juab soils. When alfalfa soil was inoculated into the same medium, the fixation was about twice that observed when virgin or wheat-land soil was used. The increase was manifested in the first-, second-, and third-foot sections of soil and was the result of either a direct or indirect action of the legume upon the non-synthetic nitrogen-fixing organisms.

The addition of ground alfalfa, ground pea vines, and ground straw to the Nephi dry-farm soil increased the nitrogen-fixing capacity during the first two years to approximately the same extent. All of the plant residues caused greater increases in the nitrogen in the soil than could be accounted for by the added nitrogen; hence the increases must have been due to greater nitrogen fixation from furnishing energy to the non-symbiotic nitrogen-fixing organisms. The greatest gains in soil nitrogen were caused by the addition of alfalfa. Some of the added carbonaceous material was still present after eleven years.

A11. Nature of Hydrogen Inhibition of the Symbiotic Nitrogen-Fixation Process. Sylvan B. Lee and P. W. Wilson, University of Wisconsin, Madison.

It has been demonstrated by Wilson and his associates that molecular hydrogen is a specific inhibitor for the symbiotic nitrogen-fixation process by root-nodule bacteria and red clover plants (*Trifolium pratense*) During the past year, experiments have been conducted in an attempt to determine the nature of the inhibiting action of hydrogen. In these experiments the pN_2 and pH_2 of the atmosphere furnished to inoculated red clover plants were varied. In some experiments, the pN_2 was held constant at partial pressures at which it is not a limiting factor in the

nitrogen-fixation process (pN_2 0.2 atm. or above) and the pH_2 was changed. In other experiments, the pH_2 was held constant at partial pressures at which it was previously found to exhibit inhibiting action (0.2 to 0.6 atm.) and the pN_2 was varied.

Mathematical analyses of the results (total nitrogen fixed) obtained from such experiments to date indicate that hydrogen is acting as a competitive inhibitor in the symbiotic nitrogen-fixation process.

A12. Some Effects of Benzoic Acid Compounds on Azotobacter Organisms. HERBERT W. REUSZER, Colorado Agricultural Experiment Station, Fort Collins.

Experiments were performed to test the effect of various benzoic acid compounds upon the flora of the soil, particularly upon the population of *Azotobacter*. The presence of these compounds brought about a great increase in the abundance of *Azotobacter* organisms. It was found, further, that benzoic acid and sodium benzoate when present in proper concentration caused not only a quantitative but also a qualitative difference in the population of *Azotobacter*. When these compounds were added to a soil in a concentration of 1 per cent, *Azotobacter chroococcum* appeared to be the only species of *Azotobacter* present. When the concentration of the compounds was increased to 2.5 per cent, a different strain of *Azotobacter* appeared which at subsequent periods of incubation displaced *Azotobacter chroococcum* entirely. The new strain was a highly motile organism forming a soluble green pigment. Its identity has not yet been definitely established, but it does not appear to have been described previously from American soils. In artificial media, the new strain is capable of growing in higher concentrations of sodium benzoate than is *Azotobacter chroococcum*. Preliminary experiments indicate that it is capable of fixing atmospheric nitrogen in the presence of considerable quantities of combined nitrogen.

A13. Factors Influencing the Bacterial Content of Aseptically Drawn Milk. KENNETH R. STEVENS AND LEWIS W. JONES, Utah State Agricultural College, Agricultural Experiment Station, Logan.

The occurrence of bacteria in samples of certified milk prompted an investigation designed to answer the following questions. What count of bacteria is really unavoidable under aseptic conditions of production? Are cows, quarters, or months significant factors in accounting for bacteria in milk produced under these conditions? What influence do these factors have on the types of bacteria present?

Samples of milk, taken from 10 healthy cows over a four-month period, were properly diluted and plated on a modified standard agar. Six replicates and a check were made on the sample of milk obtained from each quarter. Incubation was carried on at 30°C. for 5 days, followed by 2 days at 37°C. The average count per cc. of milk from each quarter of the 10 cows for the four-month period was: left front, 210; right front, 365; left rear, 157; and right rear, 224. The average counts for the individual cows over the period varied from 49 to 511.

The data were analyzed by Fisher's variance method to determine the influence of the variables on the total number and types of bacteria found. Based on the four-month average, the count showed a highly significant difference between cows, between quarters, and in the interaction of cows and quarters. The interaction between cows and months was somewhat significant, but that between months and the interaction of quarters and months was not significant. The percentage of cocci present was significant only between cows and in the interaction between cows and months.

A14. Lactobacillus thermophilus in a City Milk Supply. DAVID B. CHARLTON AND GEORGE F. STEEL, Oregon State College, Corvallis.

In view of the occurrence of unusually high bacterial counts in the milk from certain pasteurizing plants, a study was made of the thermophilic and thermoduric bacteria found in the pasteurized milk (45 milk plants) of Portland, Oregon. Isolations of bacteria from the pasteurized milk were made, and the resistance of the organisms to heat was determined in the laboratory.

Lactobacillus thermophilus was most frequently found to be responsible for the high bacterial counts noted. It occurred more or less regularly in milk from 11 pasteurizing plants. The "Standard Methods" plating technic, as employed in the city for routine control work, does not allow a good growth of the organism to take place. *Lactobacillus thermophilus* has a growth-temperature range from 36° to 62°C. The colony is not "pin-point" in size, as originally reported, when the media contains either proteose or tryptone peptone in the usual 0.5 per cent concentration. The peculiar growth requirements of *Lactobacillus thermophilus* and the fact that in various types of cultures numbers of viable cells diminish rapidly may explain why few investigators appear to have isolated it.

The organism has been isolated by one of us (Charlton) from samples

of pasteurized milk obtained in two other cities. In each case, a slight "off" odor in the milk was responsible for attention being directed to it.

A15. A Differential Stain for the Direct Examination of Milk. JEAN BROADHURST AND CHARLES PALEY, Teachers College, Columbia University, New York City.

With the stain described below, bacteria and white corpuscles are blue, the milk background is faintly pink or colorless. Higher bacterial counts are obtained than with the usual methylene blue stains (Breed, Newman) and less eye-strain is experienced.

The preparation of the stain is as follows. Dissolve from 0.6 to 1 gm. of methylene blue in 200 cc. of 70 per cent alcohol. Add 5 cc. of 1 per cent basic fuchsin (1 gm. dissolved in 100 cc. of 95 per cent alcohol). Add 3 cc. of aniline and shake well. Add 12 cc. of dilute sulfuric acid (5.7 cc. of concentrated sulfuric acid, c.p., in about 90 cc. of distilled water, then made up to 100 cc.). Mix well and filter. To every 100 cc. of filtrate, add 50 cc. of distilled water and shake well. (The directions must be followed closely. If the stain thickens on adding the sulfuric acid, dilute it with distilled water before, instead of after, filtering.)

The staining procedure is as follows. Make milk smears on glass slides as usual, 1/100 cc. of milk being spread over 1 sq. cm. (or preferably 2 sq. cm.). When dry, immerse in xylol for 1 min. and drain dry. Immerse in 95 per cent ethyl alcohol for 1 min. and drain dry. Dip slowly, 3 times, in the stain (or flood slide with stain for ½ min.) and drain dry. When thoroughly dry, rinse in water until the blue color is washed out and the smear becomes pink. Dry and examine with the oil immersion lens as usual.

A16. The Rôle of Agglutinins in the Hotis Test. ERNEST C. MCCULLOCH, State College of Washington, Pullman.

The appearance of typical yellow balls or flakes on the side of a tube of milk following incubation in the presence of 0.025 per cent of bromcresol purple has been considered diagnostic of *Streptococcus agalactiae*. We failed to obtain typical reactions with sterilized bromcresol purple milk and pure cultures, although aseptically drawn negative milk from most cows supported typical Hotis reactions with pure cultures as did sterilized milk after the addition of small amounts of most blood sera. Agglutinin absorption markedly reduced the titer for the strain used and both milk and milk-serum mixtures supported the test after heating

to 65°C., but not after 75°C., which corresponds to the thermal tolerance of agglutinins.

Our data indicate that Hotis-positive reactions will be produced by any organism which: (1) multiplies in the udder and is present in the milk drawn; (2) stimulates the production of agglutinins; (3) tolerates 0.025 per cent of bromcresol purple; (4) forms clumps, when grown in the presence of its agglutinins; and (5) produces sufficient acid from lactose to increase the hydrogen ion concentration of the clump to *circa* pH 5.4.

Hotis-positive organisms were obtained from the nostrils of Hotis-negative cows and from the throats of 76 of 84 students. The streptococci from different positive reactions or from different clumps in the same tube often differed serologically and physiologically. The technic provides a convenient method for isolating streptococci from contaminated sources, since mixed cultures of streptococci tend to segregate into homologous serological groups during agglutination by sera containing agglutinins for several groups.

A17. The Effect of Soaps on Streptococcus agalactiae. ERNEST C. MCCULLOCH, State College of Washington, Pullman.

Soaps and especially the newer types of household detergents are effective disinfectants for *Streptococcus agalactiae*. The relationship between the chemical and physical characteristics of these products and their germicidal efficiencies, coefficients of dilution and temperature coefficients are shown by graphs and charts.

A18. A Simplified Method for the Bacteriological Examination of Water. JAMES E. WEISS AND CHARLES A. HUNTER, South Dakota State Health Laboratory, Vermillion, Brooklyn College, Brooklyn, N. Y., and Kansas Public Health Laboratory, Topeka.

A simplified method for determining the potability of water has been developed and compared with the standard method. The new or "bottle" method consisted of adding 5 cc. of a concentrated broth (24 gm. of nutrient broth (Difco) to 100 cc. of water) to the sample bottle containing approximately 150 cc. of water, thus making 155 cc. of nutrient broth. After adding the concentrated broth to the water in the bottle, the mixture was incubated at 37°C. over night. The next day a loopful of broth from the bottle was streaked on a plate of eosin-

methylene blue agar. Confirmation was carried out in the usual manner.

The "bottle" method was compared with the standard method using 638 water samples. It was found that 29.6 per cent of the samples gave positive results by both methods, that 60.2 per cent were negative by both methods, and that there was disagreement in 10.2 per cent. Of the 65 water samples showing disagreement, the standard method was positive and the "bottle" method was negative in 1.9 per cent of the samples, and the standard method was negative and the "bottle" method was positive in 8.3 per cent of the samples. Thus, the difference in favor of the "bottle" method was 6.4 per cent.

Sterile bottles containing the required amount of concentrated broth were sent into the field for trial with a number of samples of water. The results were excellent. The "bottle" method proved to be much more rapid and yielded fewer false reactions. Thus, while being more efficient, it was simpler and more economical.

A19. Effect of Temperature and Test Reagents on the Voges-Proskauer and Methyl Red Reactions. REESE VAUGHN, NANCY MITCHELL AND MAX LEVINE, Iowa State College, Ames.

Of 221 strains of the colon group tested for the production of acetyl-methyl-carbinol by the 10 per cent KOH method, only 21 (9.5 per cent) were positive after one day and 26 (11.8 per cent) after two days of incubation at 37°C., as contrasted with 45 (20.4 per cent) and 48 (21.7 per cent) giving positive reactions at corresponding periods when the temperature of incubation was 30°C. The creatine-KOH reagent gave many more positive reactions. Thus, 38 (17.2 per cent) and 42 (19 per cent) were positive after one and two days of incubation, respectively, at 37°C. and 51 (23.1 per cent) after one day at 30°C. The α-naphthol reagent was most sensitive, yielding 44 (19.9 per cent) positive Voges-Proskauer tests after one day of incubation at 37°C. and 51 (23.1 per cent) after one day at 30°C. Of 198 Voges-Proskauer-positive strains tested with the standard KOH, the creatine-KOH, and the α-naphthol reagents, the positive reactions obtained one-half hour after addition of the reagents were 0.5 per cent, 52 per cent and 94.5 per cent. These figures rose to 10.7 per cent, 61.6 per cent and 99.5 per cent, respectively, after contact for one hour.

The technics recommended in "Standard Methods of Water Analysis" for the Voges-Proskauer reaction (24 to 48 hours at 37°C. with 10 per

cent KOH as the test reagent) and for the methyl red test (3 to 4 days at 37°C.) are not suitable for the detection of the maximal number of Voges-Proskauer-positive or methyl red-negative strains, and tend to obscure the correlation of these reactions. A temperature of incubation of 30°C. and the use of the Barritt α-naphthol reagent are recommended.

A20. Dye Modifications of Bismuth-Sulfite Media. HERMAN C. MASON, University of Illinois College of Medicine, Chicago.

The problem was to determine the action of dyes and certain other substances on the bismuth-sulfite medium of Blair and Wilson in order to provide supplementary procedures to those now in use for the differentiation of certain Gram-negative enteric bacilli from each other.

The following media were used: (1) Wilson and Blair's; (2) Tabet's modification of Wilson and Blair's; (3) bismuth sulfite (Difco); and (4) selenite enrichment. The dyes and other substances added to the first three of these media, in concentrations ranging from 0.003 to 0.15 per cent, were as follows: acid acriflavine, neutral acriflavine, acid fuchsin, basic fuchsin, fuchsin, brilliant cresol blue, chrysoidin, Congo red, fluorescein, indigo carmine, malachite green, methylene blue, oxyquinoline sulfate, phenol red, phosphomolybdic acid, pyronin, quinaldine red, scarlet red, sodium hippurate, tannic acid, thionin and trypan blue. A large number of enteric bacilli chosen from a variety of genera and a few other kinds of bacteria were used as test organisms. Broth and agar-slant cultures from 18 to 24 hours old of freshly isolated and old laboratory strains were employed.

The addition of the dyes produced distinctive colonies for the typhoid and paratyphoid strains. The most favorable reaction was secured by adding phosphomolybdic acid in a concentration of from 0.003 to 0.005 per cent to the bismuth-sulfite medium. The addition of such substances as tannic acid, sodium hippurate and phenol red can be adjusted to allow growth of the dysentery bacilli to take place, but this will also permit the development of other organisms.

A21. Studies on the Flora of North Pacific Salmon. JANE E. SNOW AND PAUL J. BEARD, Stanford University, Calif.

Bacteriological samples from live salmon, from salmon stored in canneries before canning, and from the sea, from the mouth of the Columbia River at Astoria, Oregon, to the upper end of Cook Inlet, Alaska, have been investigated. Six genera, *Achromobacter*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Kurthia* and *Proteus* have been

isolated consistently from all of the sources examined. The potential significance of these organisms in initiating changes leading to more advanced decomposition is discussed. The coliform group was found only in samples which had been subject to land contamination.

A22. Some Types of Microörganisms Isolated from Whole-Kernel Sweet Corn prior to Canning. MATTHEW E. HIGHLANDS, University of Maine, Orono.

A study has been made of the types and numbers of microörganisms occurring on freshly cut whole-kernel sweet corn. Samples were taken at various stages during production up to the actual canning. In addition, the cut corn was held for several hours at atmospheric temperature, during which period representative portions were plated to detect changes in the microbial population. The samples were plated on corn-extract agar and incubated at 30°C. and at 55°C. An examination of the material for the presence of anaerobic thermophilic bacteria was made. The predominant types were isolated from the plates and identified. Of the 61 cultures isolated, the following genera and species were identified: *Achromobacter globiforme, Micrococcus conglomeratus, Serratia rubra, Serratia rosea-alba, Sarcina flava, Phytomonas stewarti, Aerobacter sp., Achromobacter sp., Pseudomonas sp., Phytomonas sp., Flavobacterium sp., Chromobacterium sp.* All of the cultures except the last were identified according to the system employed in Bergey's Manual. No thermophilic organisms were encountered in any of the samples examined.

A23. Spore-Forming Anaerobes Causing Spoilage in Canned Acid Foods. C. T. TOWNSEND, University of California, San Francisco.

In recent years a number of cases of spoilage in tomatoes and low-acid fruits, such as pears, figs and nectarines, has been found to be due to a group of spore-forming anaerobes. The rods are somewhat short, with terminal oval spores. They are non-motile. The optimal temperature for growth is 30°C., but development may occur slowly at 37°C. Subsurface colonies in agar are lenticular. There is no liquefaction of gelatin. Growth with the production of some gas, but not of acid or clot, occurs in litmus milk. Indicators are reduced. The group is markedly saccharolytic, but apparently cannot utilize lactose. A considerable amount of volatile acid is produced, and the gas liberated contains approximately as much hydrogen as carbon dioxide. Growth has been noted in pear juice down to pH 3.55.

Heat-resistance studies of spores in neutral phosphate buffer and in fruit juices, using 20,000 spores per container, have shown maximal thermal-death times at 100°C. of 40 min. in phosphate-buffer; of 20, 12 and 6 min., respectively, in tomato juice of pH values 4.5, 4.1 and 3.8; of 20 and 12 min. in pear juice of pH 4.5 and 4.1; and of 10, 7 and 7 min. in apricot juice of pH 4.5, 4.15 and 3.95. Slopes of the thermal-death-rate curves (z values) have been obtained.

Use has been made of spore suspensions of these organisms in inoculating experimental packs of tomatoes and fruit juices to determine processes necessary for sterilization.

A24. The Correlation of Resazurin-Reduction Rates and Plate Counts as Indices of the Bacterial Condition of Fresh and Frozen Foods. B. E. Proctor and David G. Greenlie, Massachusetts Institute of Technology, Cambridge.

A series of comparative determinations of bacterial plate counts and resazurin-reduction rates was conducted on fresh and frozen foods, including hamburg steak, fish, crabmeat, eggs and vegetables, for the purpose of determining whether the use of redox-potential indicators offers a relatively accurate and prompt evaluation of quality.

The procedure for the preparation of the resazurin-test samples, concentrations of reagents, and the incubation temperature may be varied to advantage with different foods. Evacuation of the tubes used for the resazurin-test was found to be desirable. Filtration of certain of the food solutions to remove large suspended particles tended to increase the time necessary for reduction but appeared to give more consistent results.

Foods having high bacterial plate counts, *i.e.*, over a million per gm., had reduction rates such that color changes of the dye could usually be noted within from 3 to 5 hours of incubation. In the case of foods with very high plate counts, the reduction was sometimes almost complete in ½ hour.

The results indicate the possible application of a modified resazurin method by control officials and others interested in the detection and segregation of foods having abnormally high bacterial contents, or foods which have been improperly handled or stored.

A25. Concerning the Genera of Yeasts Occurring on Grapes and Grape Products in California. E. M. Mrak and L. S. McClung, University of California, Berkeley.

With the exception of brief studies by Holm (1908), and by Cruess (1918), the literature on the ecology and taxonomy of yeasts in California exists only as occasional references in scattered papers. The present survey was undertaken to increase existing knowledge of the occurrence and distribution of the various yeasts occurring on grapes from the principal vineyard districts of California. The study also included an investigation of the yeasts found in wines, during various stages of fermentation, which were fermented under natural conditions during the 1936 vintage. Two hundred and thirty-one cultures, representing 119 samples, were selected for study and identification. Eight genera of the perfect yeasts were represented in the 149 cultures of this group. These were *Saccharomyces, Zygosaccharomyces, Hanseniospora, Debaryomyces, Pichia, Hansenula, Zygopichia* and *Torulaspora*. In the 82 cultures of the imperfect yeasts, the 7 genera represented were *Torulopsis, Mycoderma* as defined by Leberle and by Lodder, *Kloeckera, Rhodotorula, Asporomyces, Schizoblastosporion* and *Mycotorula*. The designation of *Mycotorula* is made with reservation, because of the present confused status of the taxonomy of the *Mycotoruloideae*. In addition to observations on the ecology of the cultures isolated, certain studies were made relative to the general problem of the taxonomy of the genera mentioned.

A26. Yeasts Associated with the "Sugaring" of Dried Prunes and Figs. E. E. Baker and E. M. Mrak, University of California, Berkeley.

Dried prunes and figs commonly become covered with a white sugary substance during storage before processing and occasionally in cartons after processing. It is generally believed that this substance is composed of sugar crystals. Microscopic examinations of "sugared" dried-fruit samples obtained from all prune- and fig-producing sections in California have shown that this substance consists of a mixture of yeast cells and sugar crystals. This finding has been verified by the isolation of yeast cultures from practically all "sugared" samples.

The genera and sub-genera of the sporulating yeasts isolated were in the order of their frequency of occurrence: *Zygosaccharomyces, Hansenula, Saccharomyces, Debaryomyces* and *Zygopichia*. Several cultures of imperfect yeasts and a few cultures of bacteria were also isolated. When isolations were made from an enrichment medium containing 45° to 50° sugar, a great majority of the cultures obtained were species of *Zygosaccharomyces*. Isolations from a 15° Balling enrichment gave a

greater distribution of genera, although the genus *Zygosaccharomyces* was still found more commonly than any other.

The cultures were inoculated into a medium containing yeast extract, primary potassium phosphate, and various amounts of invert sugar. Organisms capable of developing at invert-sugar concentrations up to and including 60 per cent were predominately *Zygosaccharomyces*; those developing up to and including 50 per cent were predominately *Hansenula* and those developing up to and including 40 per cent were predominately *Saccharomyces* and imperfect types. Only one culture of imperfect yeast failed to develop in the medium containing 30 per cent or more of invert sugar. No obligate osmophilic yeasts were encountered.

A27. Observations on Certain Film-Forming Yeasts. W. V. Cruess and L. Hohl, University of California, Berkeley.

Several strains of film-forming yeasts were isolated from Spanish Sherry and from mixed cultures of Spanish Sherry yeasts obtained for experimentation on application of the Spanish "flor" procedure in Sherry making. Several other strains were isolated from mixed cultures obtained from France. The general morphology, action on various carbohydrates, spore formation, alcohol production, alcohol tolerance and various other characteristics of these yeasts were determined. The results may be stated as follows. The typical Spanish "flor" yeasts are strongly oxidative in action on the alcohol, fixed acid, and acetic acid of wine. They impart a characteristic bouquet and flavor similar to that of young wines of Spanish Manzanilla or Fino character. Alcohol tolerance for film formation is high for the fermentive types, from 14 to 16 per cent by volume, and low for the non-fermentive types, about 10 per cent. Tolerance of the fermentive types is high for sulfur dioxide; from 200 to 500 parts per million, depending on the medium.

A28. Some Lactobacilli Encountered in Abnormal Musts. Reese Vaughn and Howard C. Douglas, University of California, Berkeley.

One hundred and thirteen strains of bacteria of the genus *Lactobacillus* were isolated from samples of muscat grape musts (*Vitis vinifera var. Muscat of Alexandria*) which had spoiled while undergoing natural fermentation. These abnormal musts were high in volatile- and fixed-acid content and possessed an off-flavor, known as "mousiness". The activity of the yeasts had ceased because of the combined effects of high

fermentation temperatures and large quantities of volatile acid. Previous investigations (Vaughn) had shown that the abnormal quantities of volatile acid noted in these samples resulted from rapid acetification brought about by the associative action of yeasts (*Saccharomycetaceae*) and *Acetobacter* organisms. The lactobacilli isolated, while not producing significant quantities of volatile acid, were important because of the ability to form large amounts of fixed acid and a disagreeable off-flavor.

The peculiar "mousey" off-flavor was produced by all the strains isolated, when they were grown in grape-must or grape-concentrate media. This off-flavor has been noted by several investigators as a characteristic of certain species of *Lactobacillus*. Under the conditions of our experiments many authentic strains of *Lactobacillus* produced this off-flavor when grown in grape-musts.

Former studies on the lactobacilli causing spoilage of musts and wines have stressed the importance of the gas-forming types of *Lactobacillus*. In this investigation only non-gas-producing strains were isolated. The majority of these cultures appears to be closely related to *Lactobacillus plantarum* (Orla-Jensen) Bergey *et al.*

A29. The Fermentation of Unripe Cigar-Leaf Tobacco. D. W. McKinstry, D. E. Haley and J. J. Reid. Pennsylvania Agricultural Experiment Station, State College.

In order to escape loss from disease or frost, growers frequently harvest cigar-leaf tobacco in an immature state. Such tobacco does not undergo a satisfactory fermentation and usually is lost by spoilage during the aging process.

Investigation has shown that the fermentation of unripe cigar-leaf tobacco is characterized by the development of a Gram-negative flora rather than by the development of the characteristic Gram-positive flora of a satisfactory cigar-leaf fermentation. The number of Gram-negative rods developing upon immature tobacco has been found to exceed 1×10^9 per gm., as measured by plate counts on nutrient agar. All pure-culture isolations of the rods studied have been found to ferment the common carbohydrates, including lactose, with the formation of gas. Most of these organisms have proved to be pigment producers in nitrate-peptone broth, although the soluble green pigment has been seldom noted in other media.

Chemical investigations have revealed that the development of the Gram-negative flora upon immature cigar-leaf tobacco is associated with the following: higher content of soluble organic nitrogen in unripe than

in ripe tobacco; lower content of malic and citric acids in unripe tobacco; and the presence in unripe tobacco of reducing sugars, a type of compound almost negligible in ripe cigar-leaf tobacco.

A30. Certain Factors Influencing Nitrification and Nitrate Accumulation in Soils under White Pine Forests. JOHN M. HALE AND W. V. HALVERSEN, University of Idaho, Moscow.

The reclamation of white pine forest soils for farming has presented interesting bacteriological problems which concern the decomposition of the forest litter and the adaptation of the soil flora to a condition that will supply plant food to successive forage and cereal crops. The present study deals with: (1) methods for increasing the rate of nitrification in these soils; (2) factors which cause an increase in the number of nitrifying bacteria; (3) factors which inhibit nitrification; and (4) factors which have a beneficial influence on the rate of nitrate accumulation. Many previous researches have failed to discriminate between the nitrifying capacity and the nitrate-accumulating power of the soil.

In the present study a relative count of the nitrifying bacteria in soil has been made. The number of nitrifying bacteria was assumed to be an index of the intensity of nitrification. The nitrate-accumulating power was found by determining the amount of nitrates which accumulated in soils under a wide variety of conditions and treatments. The study showed that relatively few nitrifying bacteria exist in forest soils, but that under laboratory conditions nitrates will accumulate in such soils. This process is slow, however, as compared with that of nitrate accumulation in soils treated with nitrogen-rich fertilizers. There are substances in the forest litter which have an inhibitory influence on nitrate accumulation. These can be overcome with proper fertilizer amendments. The number of nitrifying bacteria may be increased markedly by treatment with nitrogen-rich fertilizers.

A31. Taxonomic Relationships of Certain Non-Spore-Forming Rods in Soil. H. J. CONN, New York State Agricultural Experiment Station, Geneva.

In the soil there are numerous non-spore-forming bacteria having the following characteristics: short rods, sometimes coccoid, with a tendency to be pleomorphic; Gram-negative or weakly Gram-positive; flagella sometimes absent, sometimes one, sometimes few (2–5) peritrichic, but never as a polar tuft; producing no acid from sugar except carbonic. In addition to miscellaneous soil saprophytes like *Bacterium globiforme* and

Bacterium radiobacter, this group includes the legume nodule and crown-gall organisms, and probably other plant pathogens.

Investigations show that certain species from animal sources (milk, intestines, etc.), commonly grouped in the genus *Alcaligenes*, seem to have the same general characteristics. They should not be grouped separately from the soil forms just because of a difference in habitat. Possibly some of the soil forms should be put in the genus *Alcaligenes*; but the status of this genus depends on whether its type species, *Alcaligenes fecalis*, can be recognized. Most bacteriologists recognize, under this name, any fecal organism which turns litmus milk alkaline; there is some question, therefore, whether the species can be accurately identified. This point is under investigation at present.

If *Alcaligenes* is a valid genus, it is suggested that it be placed in a family with *Rhizobium*, *Chromobacterium*, and those species of *Phytomonas* having the characteristics outlined above. For this family the name *Rhizobiaceae* is proposed.

A32. Moisture Changes and Their Effect on Soil as a Bacterial Medium. H. J. Conn, New York State Agricultural Experiment Station, Geneva.

Experiments have been conducted on sterilized soil inoculated with either *Bacterium radiobacter* or *Bacterium globiforme* after adding glucose, calcium carbonate, potassium sulfate, and ammonium phosphate. Microscopic counts of the bacteria were made 7 days after inoculation.

Five soil samples, representing 3 distinctly different soil types, were used. When the tests were made, the soils were adjusted to approximately 50 per cent of their moisture-holding capacity; but, previous to sterilization and inoculation, they had been held at various moisture levels, namely, air-dry, 25, 35 and 45 per cent, respectively, of the water-holding capacity of each soil.

The soils studied, if allowed to become air-dry, were impaired as media for the growth of the organisms, even after bringing up to the optimal moisture content and adding complete nutrients. It proved possible to restore air-dry soil to a normal condition in this respect by holding it at 35 per cent of its water-holding capacity for about 2 weeks before adding the nutrients, sterilizing and inoculating.

Experiments were carried on to show whether the improvement of soil after moistening might be due to changes brought about in it by growth of the natural soil flora during the 2-week period while the soil was moist, or due to release of alkalis during this period that might

possibly have been fixed by the soil colloids while in an air-dry condition. Indications were obtained that fixation of potassium might be one of the factors involved; but results varied too greatly to be conclusive.

A33. Variations in the Microflora of Wheat Roots Following Soil Amendments. CHARLES THOM, FRANCIS E. CLARK, M. L. FIERKE AND HURLEY FELLOWS, U. S. Department of Agriculture, Washington, D. C. and Manhattan, Kan.

To determine the effects of soil amendments upon the microflora of the rhizosphere, potted soils fertilized heavily (1) with chicken manure or (2) with chopped green alfalfa, together with unfertilized checks, were planted to wheat. The soil employed was naturally infested with the take-all fungus, *Ophiobolus*; the disease was completely suppressed in the fertilized soils. Plate counts of micropopulations in the alfalfa-treated and in the manured soil showed abrupt increases within from three to five days to maxima of six hundred million and fifteen hundred million, respectively, followed by subsequent decreases toward the count of from thirty to eighty million in the check soil. Contrasting types of microörganisms developing during the period of rapid decomposition persisted for several weeks.

A comparison of the microflora associated with the root surfaces themselves revealed high counts of from one to two billions upon roots both in untreated and in treated soils. The types of bacteria involved contrasted sharply. The majority of organisms isolated from wheat roots grown in a chicken-manured soil were Gram-positive corynebacteria, readily inhibited in culture by the addition of crystal-violet, capable of reducing nitrates, and generally requiring organic nitrogen for growth, whereas predominating types associated with roots in alfalfa-treated soils, although corynebacteria, did not reduce nitrates, but were more active in carbohydrate fermentation. Organisms isolated from roots in treated soils differed from those from roots grown in untreated soil. Manifestly, the type of amendment affects the character, if not the numbers, of microörganisms directly associated with the wheat root system.

A34. The Fermentation of Definite Nitrogenous Compounds by Members of the Genus Clostridium. H. A. BARKER, University of California, Berkeley.

The primary function of putrefactive spore-forming anaerobes in

nature is the decomposition of nitrogenous organic compounds. The object of the present investigation has been to elucidate the specific chemical activities of different species in the process of putrefaction by finding out the particular nitrogenous compounds decomposed and the products formed. This has been done by the use of anaerobic enrichment cultures containing definite nitrogenous compounds (glutamic and uric acids) as the only sources of energy. Numerous strains were isolated from the enrichment cultures and their morphological and physiological characteristics were determined.

Glutamic acid is fermented to butyric and acetic acids, carbon dioxide, ammonia and hydrogen by organisms that are universally distributed in soil. The ability of pure cultures to ferment glutamic acid is readily determined with a semisolid-agar medium containing this substance and a little yeast extract. All glutamic acid-positive strains are physiologically similar and have been identified as *Clostridium cochlearum* or *Clostridium tetanomorphum*.

Uric acid is rapidly converted anaerobically into ammonia, carbon dioxide and acetic acid. The causative organism, *Clostridium acidiurici*, develops well only on media containing urates.

The available data indicate the specificity of different organisms in putrefactive processes, the usefulness of definite nitrogenous compounds in isolating particular *Clostridium* species and the taxonomic value of the ability to ferment such compounds.

A35. Foxing and Deterioration of Paper. T. D. Beckwith, W. H. Swanson and T. M. Iiams, University of California, Los Angeles, and Huntington Library and Art Gallery, San Marino, Calif.

The deterioration of paper may be induced by a variety of factors, but the present work concerns the relationship of fungi to the process. The effects of 55 fungi isolated from paper have been studied. Sizings and fillers may further fungous growth and iron definitely is a stimulant, either in the presence or absence of sizing. The color of foxing ordinarily is unrelated to chromogenesis of the fungus but results from the nature and tint of the by-products formed. Growth of these microorganisms brings about the production of compounds which may be extracted by a solution of weak base and which are similar to humous structures in certain respects. These fungi can destroy the sheet of tissue inclusive of α-cellulose. Their metabolic demands for water are

low. An explanation for the characteristics of foxed paper is advanced therefore. Technics for the recognition of fungous vestiges in paper have been elaborated.

A36. Bactericidal Activity of "Royal Jelly" of the Honey Bee. C. S. McCLESKEY AND R. M. MELAMPY, Louisiana State University and Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, University, La.

"Royal jelly", the secretion of the pharyngeal glands of the worker honeybee which is the food of the queen, has pronounced bacteriostatic and bactericidal properties. *Escherichia coli* and *Eberthella typhosa* are inhibited by a concentration of 1–50 of the natural jelly in nutrient broth; *Staphylococcus aureus* and *Bacillus metiens* by a concentration of 1–100. The germicidal activity is markedly influenced by temperature. When 1 cc. of broth culture was added to 1 cc. of a 1–5 dilution of "royal jelly" at 43° to 45°C., the organisms were destroyed in 15 seconds; at 23° to 25°C., in from 10 to 30 minutes; while at 5°C. the organisms survived for 2 days. The bactericidal potency was rapidly reduced by neutralization of the natural acidity of pH 4.4 to 4.6. Sterilization of test cultures required only a few minutes at pH 4.6 (25°C.), but required 2 days at pH 7.0. The bactericidal capacity was largely lost when "royal jelly" was filtered through paper; it was found in the sediment after centrifugation. The active principle was removed from "royal jelly" by extraction with ethyl alcohol and with acetone. From these extracts, crystals were obtained which seemed to possess the bactericidal principle.

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PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

EASTERN PENNSYLVANIA CHAPTER

ONE HUNDRED AND THIRTY-FIRST MEETING, PHILADELPHIA COUNTY MEDICAL SOCIETY BUILDING, APRIL 26, 1938, PHILADELPHIA, PENNA.

A COMPARATIVE STUDY OF MEDIA EMPLOYED IN THE ISOLATION OF TYPHOID BACILLI FROM FECES AND URINES. *Cora B. Gunther and Louis Tuft*, Pennsylvania State Health Laboratories, 34th and Locust Sts., Philadelphia, Pa.

In this study, 2245 feces and urines were cultured for typhoid bacilli employing various media in a comparative way. Wilson-Blair's bismuth-sulphite medium was shown to be superior to Endo, eosin-methylene-blue and Leifson's desoxycholate-citrate media. The Difco modification of Wilson-Blair medium, used in these tests, is superior to Wilson's original medium, being easier to prepare, relatively more stable and more sensitive. It is useful for isolating most of the important *Salmonellas* but not for the dysentery group. Leifson's selenite enrichment medium was shown to be superior to 30% glycerine enrichment. A technic was evolved in which a loopful of feces is streaked directly on each of two Wilson plates and an emulsion also made in selenite F. After incubation, a loop of emulsion is streaked on Wilson medium. It was found that 25.5% of the total positives were positive on Wilson, direct streak, and negative after enrichment and 17 per cent were positive after enrichment and negative by direct streak. Both methods of culturing are recommended since some are missed by each technic. A change in routine technic from 30% glycerine enrichment and streaking on Endo to the technic described above has increased the positive typhoid isolations almost 50%.

THE CHEMISTRY OF A NUCLEOPROTEIN ANTIGEN —"LABILE ANTIGEN"— FROM HEMOLYTIC STREPTOCOCCI OF LANCEFIELD GROUP A. *M. G. Sevag, D. Lackman and J. Smolens*, Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

An antigen designated "labile antigen" and believed to be identical with Griffith's type-specific agglutinogen of hemolytic streptococci, Lancefield's Group A, has been described by Mudd, Czarnetzky, Lackman and Pettit (Jour. Immunol., **34**, 117, 1938). This antigen was first obtained from the aqueous extracts of streptococci disintegrated by audible sound treatment by Chambers and Flosdorf (Proc. Soc. Exper. Biol. and Med., **34**, 631, 1936). Chemical studies show that this antigen is a nucleoprotein and analyzes 2.65% phosphorus, 15.79% total nitrogen, 9–13% reducing sugar calculated as glucose and less than 2% ash. On treating with 0.5% sodium carbonate solution at 55°C for 1–2 hours, this substance is split into a protein and nucleic acid.

The protein component, on further treatment with N/20 NaOH overnight in the refrigerator and for one hour at 55°C, is rendered free of the residual nucleic acid. The analysis of this protein shows 14% total nitrogen, negative purine nitrogen, pentose and phosphorus. It shows 5.8% reducing property calculated as glucose after acid hydrolysis. Serologically, it is active with sera prepared against Lancefield's Group A and inactive against streptococcus Groups B and F. It produces immune sera upon injection into rabbits.

The nucleic acid fraction is treated with chloroform (Sevag, Bioch. Zt., **273**, 419, 1934) to remove the protein. On analysis it shows 9.25–9.67% phosphorus, 15.79–15.90% total nitrogen, 10.24% purine nitrogen, positive pentose reaction and 14.4–14.81% reducing sugar calculated as glucose.

These nucleic acids are in certain instances serologically active, depending upon the method of isolation; precipitation with mineral acids seems to destroy this property. On the other hand, precipitation with weakly acidulated alcohol seems to preserve their activity.

SOME REACTIONS OF SULFANILAMIDE WITH NUCLEOPROTEINS AND A SUGGESTED MECHANISM OF THE ACTION OF SULFANILAMIDE. *E. J. Czarnetzky* and *H. E. Calkins*, Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Hope of finding a chemical entity capable of destroying bacteria specifically without injury to the host seems to have been realized in the discovery of the action of sulphanilamide on streptococci. In order to take advantage of this finding in a search for new specifics which would combat other types of bacterial infections, it is necessary to determine the basic chemical reactions underlying the bacterial action of sulphanilamide.

There is good evidence for the belief that the surface of β-hemolytic streptococci of Lancefield Group A contains an antigen which is partly composed of nucleic acid. This antigen can be isolated in a purified form, and when treated with sodium bisulphite or sulphanilamide it becomes lytic for red blood cells *in vitro*. Yeast nucleic acid and vitamin B_1 are made hemolytically active in the same manner by both reagents. It is known that the reaction between vitamin B_1 and sodium bisulphite results in the formation of a sulphonic acid derivative of the pyramidine ring of the vitamin. Such a sulphonic acid derivative is hemolytic. A similar chemical reaction takes place between sulphanilamide and yeast nucleic acid or streptococcal nucleic acid, resulting in the production of hemolysins, which are compounds of sulphanilamide and various nucleosides.

A consideration of the toxic effects of sulphanilamide, which include hypochromic anemia and granulopenia, seems to be in harmony with the fact that sulphanilamide may react with nucleic acid compounds with a resultant formation of hemolysins.

ONE HUNDRED AND THIRTY-SECOND MEETING, PHILADELPHIA COUNTY MEDICAL SOCIETY BUILDING, MAY 24, 1938, PHILADELPHIA, PENNA.

MOLECULAR WEIGHT, ELECTROCHEMICAL AND BIOLOGICAL PROPERTIES OF TUBERCULIN PROTEIN AND POLYSACCHARIDE MOLECULES. *Florence B. Seibert, Kai O. Pedersen* and *Arne Tiselius*, Institute of Physical Chemistry, University of Uppsala, Uppsala, Sweden and the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Penna.

Molecular size and homogeneity of the protein molecules in tuberculin which elicit the tuberculin skin reaction were determined by means of sedimentation in the Svedberg ultracentrifuge, diffusion by the method of Lamm and electrophoresis in the Tiselium apparatus. Chemical analyses for nitrogen, polysaccharide and nucleic acid, and biological tests for potency, toxicity and precipitin reaction were made.

Six homogeneous molecules were isolated, one of about 10,000 molecular weight from bovine and one of about 17,000 molecular weight from timothybacillus culture filtrates. The polysaccharide had a molecular weight of about 9,000 and differed remarkably from all polysaccharides hitherto studied in exhibiting molecular homogeneity.

A homogeneous protein with molecular weight of about 32,000, isoelectric point pH 4.3 and isoionic point pH 4.7, was isolated from human type filtrate. It gave a high precipitin titer, was very toxic and produced the anaphylactic type of local skin reaction in tuberculous guinea-pigs.

PP D was separated into a mildly potent fraction with molecular weight about 9,000, an heterogeneous but highly potent fraction, and an homogeneous fraction with molecular weight of about 16,000, which was non-antigenic and found to be a stretched molecule by means of double refraction in flow.

STUDIES ON TETANUS TOXOID. *Herman Gold*, Sharp & Dohme Laboratories, Glenolden, Pa.

The injection of tetanus toxoid produces in most individuals a slight to moderate local reaction lasting 1 to 3 days. The alum-precipitated toxoid produces a subcutaneous nodule, usually absorbed in 2 to 4 weeks.

In agreement with Jones it was found that the antitoxin content was higher in individuals receiving two doses of alum-precipitated toxoid than in those receiving 3 doses of plain toxoid or plain toxoid plus 0.4% alum.

Loss of antitoxin in all persons, regardless of the kind of toxoid injected, was most marked the first few months following the initial immunization course. By the end of a year the majority of the immunized individuals showed less than 0.1 unit of antitoxin per cc. of serum—the minimum protective amount. The first dose of alum-precipitated toxoid seemed to act as "sensitizer"; following the second dose there was a prompt antitoxin increase in the blood.

The immunity response of different individuals following the initial dose series of alum toxoid is variable, making it necessary to administer a "repeat" dose of toxoid whenever an injury occurs. In most cases, five days after injection of the "repeat" dose the antitoxin titer was up to or above the protective level. Following the "repeat" dose of toxoid the protective titer was maintained for a longer period of time than after the initial course of immunization.

INTRANASAL DIPHTHERIA IMMUNIZATION IN HUMANS. *LeRoy J. Wenger, Bettylee Hampil* and *Peter Masucci*, Mulford Biological Laboratories, Sharp & Dohme, Glenolden, Pa.

Today's problem in mass immunization against diphtheria is maintaining the resistance produced by immunization. Dr. Jensen suggested (Proc. Royal Soc. Med., **30**: 1117–48, 1937) that a single dose of aluminum-hydroxide precipitated toxoid be given subcutaneously; and that the immunity produced be reinforced and prolonged by the intranasal instillation of plain toxoid four weeks after the injection.

Diphtheria-antitoxin response to single doses of alum-precipitated toxoid and of aluminum-hydroxide precipitated toxoid in same human age group were compared. A concentrated diphtheria toxoid for intranasal instillation was developed at the Mulford Biological Laboratories. Intranasal instillation in normal guinea-pigs showed, 4 weeks after 3 series of treatments, 0.5 unit of antitoxin per cc. of blood. Intranasal instillation of concentrated toxoid in guinea-pigs, 14 weeks after subcutaneous injection of a human-dose diphtheria toxoid, precipitated, showed 5 days after the last instillation more than 6 units of antitoxin per cc. Twelve young adults, still Schick positive 3 months after subcutaneous injection of a dose of alum-precipitated toxoid, were given 3 weekly intranasal instillations of concentrated diphtheria toxoid. One week later in 10 of the 12, there was an increase in antitoxin titer of from 10 to 250 times the titer before the intranasal instillation.

CONNECTICUT VALLEY BRANCH

SPRING MEETING, MAY 14, 1938, WESLEYAN UNIVERSITY

SOME FACTORS INVOLVED IN TESTING DISINFECTANTS. *Elizabeth F. Genung*, Associate Professor of Bacteriology, Smith College, Northampton, Mass.

A comparison of seven different methods of testing disinfectants, described in the literature, revealed startling discrepancies in the results obtained when identical organisms were employed by the same worker throughout. Some methods were outstanding in uniformity of results, simplicity and efficiency. It was clearly demonstrated that a chemical should not be evaluated on the basis of one test alone.

A modification of a method used for studying bacteriophage, "'phage method," was found to adapt itself to the testing of chemicals and is suggested as a preliminary test for the potential value of a disinfectant. At least 15 cc. of nutrient agar are poured into a sterile Petri plate, allowed to harden, and 0.2 cc. of the culture is spread, evenly, over the surface with a sterile bent wire or glass rod. When dry, one drop of the disinfectant is placed on the surface and allowed to absorb. The plates are then incubated under porous tops for twenty-four hours. A well defined clear area indicates by its size, freedom from colonies, etc. the bactericidal or inhibitory properties of the chemical. Blood, serum or other organic material may be added to the agar.

The results should be checked by, at least, one other method. The coverslip method, described by Jensen and Jensen (1933) is found to give uniform results as well as being more convenient to operate than the loop method, usually recommended, especially when very short time intervals are employed.

FACTORS GOVERNING THE MORPHOLOGY OF MONILIA ALBICANS. *Lynferd J. Wickerham*, Department of Bacteriology, Yale University.

A large number of strains of *Monilia albicans* obtained from various sources were studied under various environmental conditions in slide cultures.

Strains were classified into five types on the basis of the comparative number of blastospores and chlamydospores produced on the hyphae in cornmeal agar preparations. Type I cultures produced practically all chlamydospores, type V cultures produced only blastospores. These type characteristics were hereditary, but could be modified by growing the organisms on other media. On a richly nutritive medium such as glucose agar the vegetative rather than the sporulative phase was promoted, and resulted in the production of large numbers of blastospores even by strains which on cornmeal agar formed chlamydospores exclusively.

Chlamydospores were not formed in any medium at a temperature of 30°C. or higher.

EPIDEMIOLOGIC SIGNIFICANCE OF SERO-TYPING IN CHICK PARATYPHOID. *Carl F. Clancy and Erwin Jungherr.* Department of Animal Diseases, Storrs Agricultural Experiment Station, Storrs, Conn.

Three outbreaks of paratyphoid in chicks were observed in poultry stock on premises with a previous history of paratyphoid infection. The organisms obtained from the original and second outbreak in the respective cases were subjected to biochemical and serologic identification, the latter on the basis of antigenic structure according to the Kauffmann-White scheme.

In the first case *Salmonella typhimurium* was recognized as the causal agent and reisolated 1 month following the original outbreak. In the second case *Salmonella montevideo* was involved and recovered in specimens submitted 14 months later. *Salmonella oranienburg* was isolated in the third case and found again 25 months later. The occurrence in chicks of rare sero-types which have hitherto been regarded as essentially human pathogens suggests lack of species-specificity in the genus *Salmonella*. On the other hand, the recurrence of the same sero-type in repeat-outbreaks at intervals of 1 to 25 months points towards a more or less endemic occurrence of certain types, for the recognition of which sero-typing appears to be of special value.

THE GROUPING OF HEMOLYTIC STREPTOCOCCI ASSOCIATED WITH DISEASE IN CHILDHOOD. *Paul L. Boisvert.* Department of Pediatrics, Yale School of Medicine.

Lancefield has demonstrated that hemolytic streptococci can be divided into distinct groups by a precipitin test. Although human pathogenic strains usually fall in her serological group A, other groups have been found responsible for human disease.

We were anxious to obtain some idea of the frequency in human beings of infection by these other groups, especially in infants and children, in whom resistance to the hemolytic streptococcus is relatively low.

During a period of one and one-half years, all available strains of hemolytic streptococci from 248 patients, with streptococcal disease, were grouped by the Lancefield method. 217 of these patients were infants and children.

All of the 408 strains from these patients fell in group A. Hemolytic streptococci belonging in groups B, C, and G have occasionally been encoun-

tered, but there was no evidence that they were causing disease.

The findings suggest that infants and children are no more susceptible than adults to hemolytic streptococci of groups other than A. In fact, the precipitin test gave remarkably consistent results both in these young individuals, and in the adults who were studied.

A Comparison of Properties of Toxic Extracts of the Eberthella-Salmonella Group. *Eleanor Cornell Bower.* Smith College, Northampton, Mass.

Toxic extracts have been prepared from *Eberthella typhosa, Salmonella enteriditis, Salmonella aertrycke, Salmonella schottmulleri,* and *Salmonella suipestifer* by holding washed suspensions of 18-hour beef-heart infusion agar cultures at 58–60°C. for 4 hours, centrifuging, and filtering the supernatant through Berkefeld N or W candles. These filtrates, when injected intraperitoneally, would kill 3 out of 5 twenty-gram mice in doses ranging from 0.3 to 0.8 ml., depending on the organism used in preparing the extract. All extracts suffered some diminution in their lethal potency after heating at 100°C. for 1 hour, although the same amount of extract which, unheated, would kill 3 out of 5 mice in 24 hours, still retained the ability to make the injected animals ill.

The extracts had the same physiological effect on injected mice, differences observed being quantitative rather than qualitative. At autopsy, the most striking and typical finding was an involvement of the small intestine which was empty, transparent in the duodenal and jejunal regions, and filled with a yellowish fluid.

Specific agglutinins for both O and H antigens were obtained by injecting rabbits with small graded doses of the toxic extracts. The antisera did not possess any very potent antitoxic immunity in so far as this property could be demonstrated by passive protection experiments with mice. Antisera injected in 0.5 ml. amounts one-half hour prior to the administration of 2 minimum lethal doses of extract seemed to increase the average time to death, but the animals were always ill and the percentage of survivors never very high. Homologous antisera gave the best protection. Heterologous antisera to extracts of organisms containing common O fractions, while less protective than homologous sera, seemed to have more antitoxic potency than antisera to extracts of organisms not sharing antigens in the soma.

Variation Studies of Mycobacteria: Colonial and Cellular Morphology. *Harriette D. Vera.* Department of Bacteriology, Yale University.

Four human and two bovine strains of the tubercle bacillus, one strain of *Mycobacterium smegmatis* and several strains of soil mycobacteria were grown under various conditions for different periods of time. They were cultured on Long's agar medium and on meat-extract agar. They were grown in atmospheres containing varying proportions of oxygen, nitrogen, and carbon dioxide. Careful records, including photographs, were made of the colonial characteristics.

Growth and pigmentation of colonies were increased by an increased oxygen supply. When the oxygen supply was restricted, the colonies were white or colorless, and they tended to be somewhat more smooth than those grown in the presence of air.

Approximately 300 colonies were

embedded in paraffin and were sectioned vertically, 2–4 micra thick. Sections of all colonies were stained by the Ziehl-Neelsen technique; sections of most of the colonies were also stained by the Gram method. No section of any colony was seen that was composed exclusively of acid-fast cells. The proportion of acid-fast cells, as well as their number, appeared to be increased by a high-oxygen atmosphere. Almost all colonies showed stratification in the dye reactions and in the cellular arrangement; the stratification pattern, however, was variable. The positions of the Gram-positive substance and the acid-fast cells corresponded closely, but were not identical. Very short coccoid forms occurred in colonies grown on meat-extract agar.